INVESTIGATIONS INTO THE MORPHOLOGY OF THE RAY FUNGI

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BY

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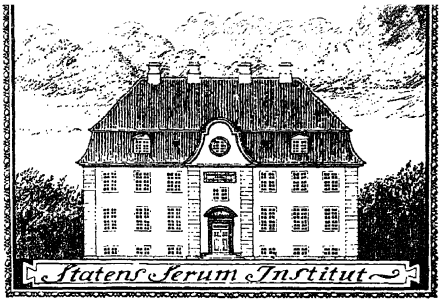

LEVIN & MUNKSGAARD PUBLISHERS
COPENHAGEN :: MCMXXIII

Denne Afhandling er af det lægevidenskabelige Fakultet antagen til offentlig at forsvares for den medicinske Doktorgrad.

København, den 24. Oktober 1922.

C. Rasch
f. T. Dekanus.

The present work has been performed at the State Serum Institute of Copenhagen. I desire to express my best thanks to the Chief of the Institute, Dr. THORVALD MADSEN for the excellent facilities for working which the Doctor takes always a great interest in procuring for his assistants, and for the personal interest and encouragement with which he has followed my work.

At the same time, I offer my best thanks to the various Institutes through the kind offices of which I have obtained various fungi for examination.

Printed in Denmark.

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A/S Oscar Fraenckel & Co s Bogtrykkeri. København.

INTRODUCTORY REMARKS

It was first with *Robert Koch's* ingenious invention of the solid nutritive medium, the primary condition for pure cultivation, that the work for classification of bacteria was led into a straight track.

When pure cultivation was made the basis of all bacteriological research, it was soon discovered that the previously supposed lawlessness of the morphological and physiological phenomena encountered in the study of bacteria, could be replaced by a system with even very definite boundaries. It was soon found that, morphologically, the bacteria could be divided into some few groups, their being no question of transition from group to group.

The glorious results attained in bacteriological science are to a great extent due to this constancy in morphology and accordance in physiology displayed by the various pathogenic microbes when pure cultures are worked with, and, it is therefore not surprising that bacteriologists who had been through the first difficult days of the bacteriological era, were disposed to hold energetically to these fixed boundaries, and showed great reluctance to attach any importance to the records, which appeared now and then, of morphologically divergent forms of bacteria.

The classes into which the bacteria were divided, and are divided to the present day, are the following: the rod-shaped, the spherical, and the forms which represent smaller or larger parts of the curves of a spiral; the reproduction of these various forms occurs simply by cleavage into two equal parts along a transverse axis, as soon as a young individual has grown to about double its normal size.

However, as mentioned above, instances were recorded from

various sides of findings of bacterial forms which were not naturally classifiable within this narrow system; and, moreover, any bacteriologist will, in the course of his work, occasionally encounter forms which differ essentially from the normal.

These variations of bacterial forms did however usually arise under conditions of life that would be considered unfavourable for the development of the specific microorganism under observation, if, by no other reason, then just by virtue of the circumstance that such atypical forms arose. A collective designation for all such atypical forms was soon introduced by *Buchner*, namely »involution forms«. By this designation was thus meant a form, the occurrence of which was due to unfavourable conditions of development. There is but little doubt that this interpretation is correct in regard to numerous of the atypical forms encountered in bacterial cultures, this view being moreover borne out by the fact that the divergent forms also show other signs of decay or degeneration; but, in addition to these degeneration forms, in regard to which all investigators doubtless agree, descriptions have been published in the course of times of numerous other divergent bacterial forms which can hardly be enlisted under the collective term »involution« forms, and, while bacteriological science cannot be said to have acknowledged these forms as being on a par with the so-called normal forms, bacteriologists who have personally studied these peculiar bacterial forms, almost unanimously agree in that they should be conceived as natural phases of development of the bacteria concerned, and, also that they are of quite special interest, since the study of them might supply us with some information as to the origin of bacteria and their natural position within the botanical system.

A group of bacteria which has more especially afforded ground for speculation of this nature, is that of the »*branching bacteria*«. It was known rather early in the bacteriological era, that among the bacteria there were such as would now and then display »true branching«, that is to say, were apparently able to multiply in a way that would not fit in with the once adopted system, while such a mode of reproduction was well known from other microorganisms for which it was the normal one.

Such »branchings« were first demonstrated in as far as the diphteria bacillus was concerned, by various observers, and very soon statements of similar findings were advanced by other workers

in regard to the tubercle bacillus, the lepra bacillus, and several others.

These findings afforded ground for much speculation and many theories — as a rule but poorly founded — as to the possible relationship between these bacteria and some of the higher fungi, and gave rise to the view held by many bacteriologists that these forms of microorganisms should be excluded from the groups of true bacteria to be classified with the morphologically higher differentiated fungi, while others were inclined to place these branched organisms into the large heterogenous crowd of »involution« forms.

The author has previously been engaged in studying such variations of bacterial forms, and found such among them in regard to which the designation involution forms was utterly misleading. During that work I also touched upon the problem of the significance of the branching forms, without being able to contribute to its elucidation.

More recently, however, I encountered a microbe with richly branching filaments which, according to its general morphology, must be classed with the group of fungi generally known under the name of Ray Fungi. When cultured on artificial media, this fungus exhibited a strange transition to what is usually called the bacillary shape, almost totally losing its side branches. This of course roused my interest for the whole question of the significance of bacterial ramifications and as, afterwards, I succeeded in devising a simple method for direct observation of bacterial growth on solid media, it became natural to compare the growth-forms of the named branching fungus, partly with previously found ray fungi, partly with various members of those bacterial species which most commonly display the phenomenon of true branching.

These investignations soon proved that, by means of the named method, it was possible to get a much better general view of the morphology of the microorganisms under observation than that afforded by the usual methods of examination; and, since already during the initial stages of the work there appeared among the microorganisms at my disposal such variations of form as were, partly not commonly known, partly not *correctly interpreted*, my investigations were naturally extended to comprise as great a number as possible of all sorts of branching microorganisms belonging to the group of fungi which goes under such different

names as Discomyces, Actinomyces, Streptothrices, Micromyces, Nocardia, Oospora, and several others still.

These microorganisms showed among their representatives such constant differences of morphology that it became feasible, on the basis of these, to classify them into groups of mutually interrelated members.

Finally, a comparison was undertaken between these fungi and the so-called branching bacteria with the object of finding out, as accurately as possible, what points of resemblance, and what points of difference, in respect of morphology, actually exist between them, and, eventually, on the basis of these findings to form a judgment on the possible relationship of these bacteria to the above-named branching microorganisms.

Besides the purely morphological investigations, I have included in the present work those physiological investigations, only, which have a direct bearing on the determination of the nature of a morphological feature.

METHOD OF TECHNIQUE

One need not have been long engaged in bacteriological research before one is struck by the great variations displayed by bacterial colonies, even though they be constituted of elements which, in ordinary smear and emulsion preparations, look exactly alike.

One bacterial species consisting of short rod-shaped organisms will, for instance, form flat smooth circular colonies; another, constituted of quite similar elements, will form dry »humpy« and irregular colonies on the same medium.

We know very little as to the causation of these variations, and only few works within the bacteriological literature have treated these problems more elaborately.

A question that comes in here, is that of reproduction. Do bacteria always multiply in the generally accepted way: by transversal division into two individuals, as soon as a young individual has increased in growth to about double its normal size? Does reproduction occur in the same way whether the cultures be young or old, and whether it take place in the centre or in the periphery of the colonies? Can the possible power of motility of the bacteria, on solid media, be thought to be of significance for the forms of the colonies, and what part do the bacterial capsules play in this respect? All these relations, and many others, such as, for instance, the chemical action of the medium, must be imagined anticipatively to play a part for the structure and consistence of bacterial colonies.

The usually applied methods, stained emulsion and smear preparations, give us but poor information on these points; these coarse methods have, partly, the disadvantages that coherent organisms may be detached from one another, the bacterial forms may

be totally changed by the staining and fixation processes, and, as a rule, they yield very deficient knowledge as to the arrangement of the bacteria in the colonies. And, furthermore, the preparations obtained by these methods allow us but a poor general view of *all* the forms found in a colony and their quantitative distribution.

Other features, which it is difficult to get elucidated are, for instance, the origin and future fate of the »atypical« forms of bacteria.

By carefully repeated studies we may of course form a judgment as to the mode of formation of these forms and as to what becomes of them, but very often we have to content ourselves with suggestions, because it is, in fact, extremely difficult to reconstruct the course of development of a colony. And the more polymorphous the picture is, the greater becomes the difficulty of the task, and still more so when the question is of microorganisms having a definite cycle of development.

Let us, for instance, imagine a microbe whose development is constantly the following; first, it forms a unicellular asteriated mycelium, which however early divides into minute short rod-shaped, loosely connected segments. Examined in the usual preparations, such a microbe would readily produce the impression of being a minute irregular rod-shaped organism with a tendency to form perhaps now and then small side-branches, and it would doubtless be classified among the rod-shaped bacteria, with the supplementary remark that it presents a tendency to formation of side-branches, which would, moreover, probably be designated as involution forms.

In order to solve some of the above named questions, we should be able to directly follow the formation of a colony step by step from the very beginning.

Are there methods of technique applicable for such a purpose?

On perusing the large handbooks, for instance *Kolle & Wassermann, Friedberger & Reiter:* Die allgemeinen Methoden der Bakteriologie, 1912, we find, of methods for direct observation of bacterial growth, only the soon very ancient mode of examination in »hanging drop preparation of broth culture«.

Other methods have, however, been devised, but they have not been very extensively used to judge from the number of works in which we find them applied.

Of such methods for direct observation and tracing of bacterial

growth and colony formation, one of the best known is *Burri's* Indian ink method.

While working out his ingenious method for the isolation of bacteria in pure culture from one single cell, he found that it was also possible to follow the bacterial multiplication in the minute Indian ink droplet on the gelatine plate.

The first phases of bacterial development are also distinctly visible under a system of high-power dry lenses and likewise under oil-immersion lenses, but, as soon as some more elements have been formed, some of these will push in beneath the Indian ink, and the image becomes blurred. Another drawback in the Indian ink method is that gelatine is unfavourable for the growth of many species of bacteria, nor is Indian ink a quite indifferent substance, which appears from the fact that several bacterial species will not resist emulsion in Indian ink, so that *Burri's* method is inapplicable for these.

Scheibert has modified *Burri's* method, for the purpose of investigating the formation of colonies on agar, by spreading the Indian ink containing the bacteria in a thin layer over this medium. The above named drawbacks would however be supposed to apply also to this mode of procedure.

Besides, *Hill* has evolved a good method, the principle of which is the observation of the bacteria in a moist chamber, the hanging-drop of broth culture being substituted by a small »hanging-block« of agar, excised out of a plane agar plate. The surface of this small agar cube touching the cover glass is inoculated with the organism to be examined, and the examination is undertaken by means of immersion lenses.

A very great advantage of this method is that it enables the observer to simultaneously follow the growth of all the elements on the agar surface in a relatively easy manner, because they are fixed to same. (Motile bacteria, however, will often be seen to move lively about in the small layer of moisture which is formed between agar and cover glass and thus disturb the examination).

The conditions under which the formation of colonies takes place here, are of course different from those of the uncovered agar plate, so that no direct conclusions can be drawn for colony formation.

Other investigators have, like *Hill*, substituted solid media for the hanging drop of broth in the moist chamber, but in contradi-

stinction to *Hill*, they have inoculated the lower surface of the quite thin layer of medium; *Bergstrand*, for instance, has followed the growth of some diphtheria bacilli on *Löffler's* serum in this way.

The only one of the named methods for direct investigation into bacterial morphology and development that has been commonly used, is the »hanging-drop« method. This is also an excellent method for the investigation of many of the morphological phenomena, whereas it is inapplicable for the examination of others, such as, for instance, — which is immediately evident, — those of colony formation. And, even for examining the growth of single bacteria, the method may fail, more especially in the case of motile bacteria. Moreover, the method is rather troublesome to work with, which of course plays an essential part when the question is of numerous examinations.

As the method described below in reality forms the basis of all my investigations, I shall enter a little more into details about the way in which it was evolved.

Originally engaged in investigations into the morphology of a quite different group of organisms from the one dealt with in the present work, I had, at a given time, to undertake pure culturing from single bacterial cells, and, for this purpose, I became intimately aquainted with Burri's Indian ink method, the simplicity of which will compell admiration from any one who works with it.

The only thing which seemed to me a little unpractical in this method, was the deposition of the Indian ink droplets on the gelatine surface of a Petri dish, because this unhandy dish is in one's way all through the progress of the examination.

Therefore, I chose to pour a thin layer of liquid gelatine on to a previously sterilized slide, upon which the Indian ink droplets were then deposited, for instance in three rows at proper intervals. It was now extremely easy quickly to examine alle the black spots when the slide was fixed on the mechanical stage of the microscope, and to mark out the one, or several, which contained one single cell only.

As mentioned above, *Burri* claims applicability of his method also for examination of bacterial multiplication, and, with this slight modification, I thought to have obtained a serviceable method for following the development of the bacteria. However, as already referred to, the sharply defined initial picture occurring when the

bacterium is wholly surrounded by Indian ink, will become quite blurred as soon as the new-formed elements push in beneath the Indian ink and cause it to be totally broken up.

In view of this complication, *Burri* indicates that the images will become more distinct, if the Indian ink be somewhat more diluted than in the case of pure cultivation, which is also correct, for: the higher the dilution of the Indian ink suspension, the easier can the growth be traced. The next step was, of course, an attempt to do quite without the Indian ink and just to smear the bacteria in a thin layer on the gelatine surface.

To my great surprise it actually proved easy to distinguish the single cell on the gelatine surface, without the contrast of Indian ink, with a high-prower dry lens, provided the light was good.

Gelatine being however no particularly suitable medium as far as most bacteria are concerned, the next step was to examine whether the growth could be followed with the same ease, and equally distinctly, on an agar plate, as on the gelatine.

This proved to be the case! First I applied a similar mode of proceeding as with the gelatine. The agar was melted and, in a very hot state, poured over the sterile slides by means of a Pasteur pipette, and the bacteria were inoculated in a streak across the agar. In this way it was easy to detect a spot where the bacteria were distributed in suitable number.

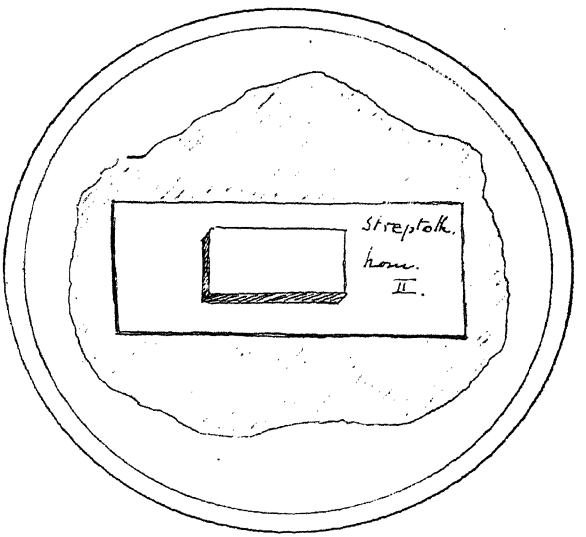
Instead of pouring the agar on the slides, by which it was difficult to obtain a perfectly level surface, I afterwards proceeded to excise suitably sized cubes out of the agar plate in a Petri dish by means of a knife, previously sterilized in a flame and cooled. These agar cubes, the planes of which should be parallel, and which should preferably not exceed 3—4 mm in thickness, are now deftly lifted on the blade of the knife and placed on a previously flamed and cooled slide, leaving the borders of same fairly free.

For convenience sake, slide + medium will in the following be termed agar-slide. The agar is now inoculated in a streak with the bacterial species, the examination of which is desired.

Using a weak-power dry lens system, and focussing on the agar surface, it will always be possible to discern the fine streak formed by the inoculation needle. The microscope is now adjusted to high-power magnification. (The objectives I employed in this work were strong apochromates of 3—4 mm. focal width, the

magnification being 7—800 times with Zeiss ocular no. 4). A spot where the bacteria lie suitably scattered can now readily be found; the best possible light is procured by using a very powerful metal filament lamp with frosted bulb of for instance 100 candle-power and shutting off by means of the diaphragm (how much depends of course on the thickness of the layer of agar).

Provided light and adjustment are as they should be, young bacteria will appear as strongly refractive, sharply defined bodies, easily distinguishable from adventitious contaminations of the agar surface.



Having found a place where the bacteria are scattered at suitable intervals, the organisms within this field of vision are drawn as accurately as possible, and, by means of the scales of the mechanical stage, the area is marked; the agar-slide is now removed to be placed in a Petri dish at the bottom of which is a piece of moist filter-paper, this dish being then incubated at the temperature at which it is desired to follow the growth (See Fig. 1).

The selected area of the agar-slide is now examined at proper intervals, and the changing images drawn. In this way it is possible simultaneously to follow the development of several bacteria within

the same field of vision, and to see how the young colonies are formed.

The larger the colonies grow, the more difficult is it to trace their growth, and recourse must often be had to special devices, as will be stated below in the respective sections.

As for the further development of this method for application in pure cultivation, I shall refer to my paper in Hospitalstidende 1922, p. 86, or Journal of Bacteriology 1922, p. 537, in which there is moreover an elaborate description of the method devised by *Hort* for pure cultivation of bacteria.

The question will no doubt arise whether this method is not compromised by infection from the air. To this I shall say, that it may of course happen that a germ descending from the air settles and develops into a colony; the significance of this is however surprisingly slight, for, on following f. inst. the growth of a culture at a few hours' intervals through some twenty-four hours, in most cases only one isolated, or, at most, a few easily discernible colonies arisen from germs from the air, can be observed, even though the agar-slides had been exposed to contamination from the air for about 10 minutes at a time. In my own case, I do not recollect to have had one single experiment spoiled on account of »alien« microbes from the air having settled on just that spot of the agar surface which was subject of examination.

With the technique here applied, the agar cube will keep its shape witout perceptible dessication for several twenty-four hours, the bacteria growing equally well under these conditions as on the agar plate in Petri dishes.

If the question is, only, to form a coarse survey of the appearance of the colonies and the morphology of the individual elements within them, the mode of proceedings may be the usual one of inoculating the agar plate in a Petri dish, for instance by smearing the bacteria across same by means of a glass rod, bent at an angle, and, at certain intervals, excising cubes of the agar and examine same on the slides, which, in this case need of course not be sterile.

By continuous inspection one obtains in this way an excellent survey of what happens in the colonies. Then, if among the elements some are found which call for closer investigation, the above mentioned measures are proceeded to. As for the accurate mar-

king of the selected field of vision refer to above mentioned paper in Journal of Bacteriology.

If some details are difficult of observation with high-power dry lenses, good information is often obtainable by placing a cover-slip on the agar cube and examine under immersion lens. By this means, the bacteria stand out as larger transparent bodies with sharply defined outlines, and one has an image of the morphology of the bacteria which leaves nothing behind in respect of distinctness when compared with the best stained films, possessing moreover the advantage that the bacteria usually appear much larger than in the fixed preparations..

As will appear, the method here described is extremely simple and uncomplicated, so that it seems surprising that it has not long ago been evolved and applied side by side with the hanging-drop method, to which it is superior in many points. If the bacterium in question is unable to grow on agar unless certain substances are added, such as serum, ascites, haemoglobin, salts etc., these should be added to the melted agar, and the inspection undertaken in the usual manner. An indispensable condition being that the medium should be clearly transparent.

Not until late in 1920, when I had applied the named method in its ultimate form for some time, there appeared an English publication by *Hort*, who had been led to devise a very similar method in his endeavours to improve the known methods of isolation of single cells for pure cultivation.

Like I did to begin with, he pours the agar out on sterilized slides, inoculates same and covers it by a thin slice of celluloidin provided with small holes at certain intervals. These holes are covered by cover-slips. The examination is undertaken with high-power dry lens.

By means of the named procedure, it will no doubt be possible, in most cases, to obtain a true to lift picture of the morphology of a bacterium and of its development and colony formation.

Investigations into the growth of anaërobic bacteria may also be carried out without difficulty by application for instance of the method devised by *Mc. Intosh & Fildes* for cultivation of anaërobic bacteria.

Where other procedures adopted to the special requirements of the tasks in hand, have been applied, these are mentioned later in their proper connection.

HISTORICAL INTRODUCTION

It is not my intention to attempt giving a record of the historical development of our knowledge of that group of fungi which is usually united under the generic term: Ray Fungi; moreover, the result of such an attempt would no doubt be rather meagre, since our knowledge of these fungi is in many respects still very incomplete — not least in respect of morphology — in spite of the almost overwhelming number of works treating of this and related subjects. Not even the most thorough study of the literature would yield an efficient basis for the understanding of the botany of these fungi.

It is clear that among this abundant literature there are several works which bear witness of great skill and profound study, and, if one has personally devoted a close study to a series of different fungi, it will be found, by careful perusal of the literature, that most of the morphological features have been correctly described, that is to say isolated correct observations can be found spread about in many works, while they have unfortunately never been collected into one complete work. On the contrary, a hard fate seems to have involved these problems from the moment when the first representative of the ray fungi was found; one might almost venture to say that the more one occupies oneself with the works extant, the more confused will the morphological picture of the various ray fungi appear, and the more difficult will it become to take up a definite standpoint in the often rather violent controversies as to the significance of one or other morphological feature.

I shall therefore defer a more thorough review of the more important papers until I have described the fungi examined in the present work; because a comparison and valuation will then be much more fruitful than at the present stage.

I shall here just quite briefly mention those works which have contributed the most to our knowledge of the ray fungi, and, at the same time, try to point out some of the reasons why we have not arrived at a clearer understanding as to the structure of the various fungi.

The botanist, *F. Cohn* (1873) was supposedly the first to recognize that he had to do with a hitherto not identified species of fungus, when examining some organisms, which made up a concretion from the lachrymal duct, sent to him by *R. Foerster.*

According to his description, there can be no doubt that the organism in question was actually a ray fungus. Unfortunately, the ramifications were interpreted as false branching in analogy with the »branching« known by *Cohn* from his recently discovered Cladothrix dichotoma, and this had the effect that the fungus — which failed to grow — was classified among the bacteria under the name *Streptothrix,* together with the Leptothrices and Cladothrices which, as is well known, do not form true side branches.

This meant two mishaps at one blow: firstly, the fungus at issue was correlated with a group of fungi with which it had nothing at all to do — the effect of which soon manifested itself — and, secondly, as appeared later, the name Streptothrix had already in 1839 by *Corda* been given to a quite different group of fungi, which had nothing to do with the ray fungi.

In the time following, a number of various similar fungi were discovered, but, owing to *Cohn's* classification of the Streptothrices among the bacteria, similar statements as to false branching in the new found fungi persisted to appear, even though the illustrations of the said fungi often show, with perfect distinctness, that there is nothing »false« whatever about the side branches. Indeed, up to quite recent works, we steadily encounter long discourses upon the differences between true and false branching as a reminiscence of *Cohn's* first description, and, as late as 1913, *Petruschky* classes the ray fungi in groups correlated with the Cladothrices and Leptothrices.

Bollinger, in 1877, from a case of cattle actinomycosis, isolated a delicate network of filaments in the »granules«, which were sent

to the botanist Harz for investigation; he found that the organism was a not previously described fungus with a delicately filamentous mycelium with true branching, to which he gave the name *Actinomyces bovis.*

The same ill luck, as named in the case of *Cohn,* also occurred with Harz, since the name Actinomyces had long ago been used to designate another fungus discovered by *Meyen.*

Shortly afterwards, (1878) *J. Israel* found similar branching threads in actinomycosis in man, to which he gave the name Actinomyces hominis.

It was not until much later that success followed the attempts at cultivating these fungi.

In 1888, *Nocard* found the microbe which is responsible for the disease usually called »Farcin du Boeuf«; but the ramifications of this fungus — which we now know to be true branching — were also by him interpreted as false branching, which made him classify his fungus among the Cladothrices. Otherwise, he states that this organism usually appears in the cultures as minute rods, resembling diphtheria bacilli, and that it forms spores!

In order to get away from the unfortunate nomenclature inaugurated by *Cohn* and *Harz, Toni & Trevisan* in 1889 proposed to name the whole group Nocardia after Nocard, a proposal which found but slight response until it has been adopted more recently by some French and English authors.

Affanassiev & Schulz (1889) have doubtless also obtained pure cultures of ray fungi; the description in the only accessible work is however too incomplete to give one a clear idea as to the morphology.

In Almquist's work, (1890), in which he describes three saprophytic ray fungi, we encounter for the first time cultures of ray fungi hich produce aerial hyphæ, which later divide into spores that are again able to develop into a unicellular mycelium.

The nature of these ramifications is corectly described, but nevertheless *Almquist* classifies these fungi with the bacteria, that is to say with the pleomorphic group.

I 1891 *Eppinger* discovered a branching fungus, in regard to which a most beautiful illustration seems to leave no doubt as to the nature of the branching, but *Cohn's* influence is still so strong that he takes the side branching to be false and names the fungus Cladothrix asteroides. In older cultures this fungus divided

into small segments, which *Eppinger* thought were motile; no later investigation has however corroborated this latter view.

Two very valuable works appear now almost synchronously (1891).

Independently of one another, *Bostrøm* and *Wolf & Israel* succeeded in obtaining pure cultures of fungi from actinomycotic affections in cattle.

These pure cultures of fungi displayed however conspicuous differences. *Bostrøm's* fungi, which preferably grew aërobically, formed long branching threads and after growing for some time on solid medium, the cultures became covered with a white »powder« of »spores«; while *Wolff & Israel's* microbes grew much better anaërobically, appearing in the cultures almost exclusively as rods suggestive of those formed by the diphtheria bacillus, and did not form spores.

This discrepancy between the discovered microbes, which either party had good cause to regard as the etiological agent of those cases of actinomycosis from which they had been isolated, gave rise to many disputes, and it appears distinctly from *Wolff & Israel's* work, that these authors utterly fail to understand *Bostrøm's* statements and to make them fit in with their own findings. And feeling convinced, on the basis of successful animal experiments, that their own microbe is the true cause of the disease, and as *Bostrøm* had not been able to produce experimental actinomycosis with his fungi, they felt further persuaded that there must be some error or other in *Bostrøm's* investigations.

The idea that two so totally diverging fungi should both of them be able to produce a disease with so typical a pathological aspect as actinomycosis, did not occur to them.

This controversy as to the nature of the causative microbe in actinomycosis, has been continued up to our own days, and it cannot be said that the problem has found a satisfactory solution. (cf. footnote).

The greater the number of investigations performed, the more difficult has it proved to be to delimit the disease called actinomycosis, and the greater number of different microorganisms have appeared to be able to cause affections which pathologico-anatomically resemble each other.

We shall come back to this question again in a later chapter on the growth-forms of the ray-fungi within the animal organism.

We are indebted to *Sauvageau & Radais* for an excellent work on some ray fungi discovered by them, the development of which they follow thoroughly step by step.

They saw clearly that essential differences prevailed between the microbes examined by them and the *Wolff-Israel's* fungus, and emphasized that these various fungi cannot be classified near to each other in the botanical system.

S. & R. committed however also the almost traditional error in their endeavour to make the nomenclature more clear, in that they gave the name Oospora to those ray fungi which formed an aerial mycelium, and which they reckoned to the Hyphomycetes, a name which had already been given to another group of fungi examined by *Wallroth;* and, later investigations have shown that these two groups have nothing in common.

Another work in which the morphological investigations are excellently carried out, is that of *Vincent* (1894) on the fungus which is the cause of the so-called »Madura foot«. This work was the more significant inasmuch as he was the first to cultivate this fungus. He names it Streptothrix and describes it as a mycelial fungus, which forms aerial hyphæ that again divide into spores.

Vincent has in fact left very little for later investigators to add to his description of this fungus.

It is strange to notice in the literature how long time it may take for a previously made experience to gain ground and be understood by new investigators. In numerous works on ray fungi we may encounter the remark that older cultures will often assume a sort of »chalky« or »powdered« appearance, and that this »powder« consists of small elements, usually oval or round in shape, which by most authors are interpreted as a kind of spores. The deeper understanding as to the mode of formation of these »spores« is however often lacking, in spite of its having been shown, for instance by *Vincent,* that this white powder is actually formed by aerial hyphæ, and moreover, direct examination consistently shows that it is the aerial hyphæ which cause the white layer to appear. For instance, in 1914, in his treatise in *Kolle & Wassermann's* Handbook on the Madura fungi, *Babes* mentions this »powder« in a way which clearly shows that he has not understood *Vincent's* work correctly, inasmuch as he says: »In älteren Kulturen bestehen die Kolonien aus einem ziemlich dünnen Fadenpilz, oft aus kokkenähnlichen Gebilden zusammengesetzt mit

der Tendens in kleinen diplokokkenähnlichen Stäbchen zu zerfallen. Dies berechtigt aber nicht, von Aktinobakterien zu sprechen, wie es Lignières für derartige Formen vorschlägt weil ja auch höhere Pilze (Favus) derartige Formen aufweisen«, to say nothing of the fact that Lignières means something quite different when he talks of »Actinobacteria«! And, in similar ways, we find this »powder« referred to in numerous works up to as late as 1914, when for instance *Rodella* seems surprised at its occurrence.

This is of course due to the circumstance that the usual technique of examination, which is quite good where the question is of ordinary bacteria, does not suffice when the objects under observation belong to the morphologically higher differentiated fungi.

In 1896, in *Flügge's* Handbook on microorganisms, *Kruse* undertakes a correlation of the works extant at that time, uniting all the species described into one group, which he claims to be closely allied to the bacteria in the botanical system.

A treatise by *Olsen* (1897) shall just be mentioned, not on account of its creditable sides, but rather to be warned against, since it will only serve to confuse the experiences gained, owing to a number of venturous inferences by way of analogy, which are however not based on thorough investigations.

Berestnew in 1898. gives a description of some various ray fungi, showing these to be of very common occurrence in nature, more especially on grasses.

Lachner-Sandoval (1898) took upon himself the task of correlating the literature hitherto extant, giving a fairly good account af the botanical side of the question up to that time. In addition, he gives a very elaborate description of a Streptothrix albido-flava, which he found especially adapted for closer morphological study, because it had not gone through any parasitic stage.

This organism proved to be a ray fungus of a morphology resembling very much that of f. inst. *Vincent's* Madura fungus, the description giving nothing especially new, while the author excellently accounts for some previously made experiences.

Lachner-Sandoval is however to blame for one thing: he draws too wide conclusions from the investigation of this single fungus, inasmuch as he takes for granted that all the previously examined ray fungi should be morphologically identical with this one. Of value is his emphatic demonstration of the differences between the examined ray fungus and the true bacteria.

Among other things he writes in regard to the morphology: »Das Aussehen der Kulturen von Strahlenpilzen überhaupt ist so charakteristisch, dass man sie dadurch leicht von den Bakterien auseinander halten kann, dagegen bieten die einzelne Arten unter sich wenige bemerkenswehrte Unterschiede.« That this is not so, he might have been enlightened from the available literature.

Many other investigators, like *Lachner-Sandoval*, are apt to draw too wide inferences from the investigation into the morphological relations of one single fungus. And this has not seldom led to rather violent contests in which both parties miss the mark, because they talk of different things, and not, as they believe, of the same. Such is, for instance, doubtless the case in the dispute between *Gilbert* and *Neukirch*.

Silberschmidt (1901) describes various fungi he has discovered, and proposes a division of the various species into two groups, according to whether their colonies can be easily split mechanically, or not. He supplies no information as to the possible underlying cause of this distinguishing feature.

In this connection I shall just mention the work by *Lignières & Spitz* (1904), because these authors propose a classification of the ray fungi with special reference to those forms which may cause actinomycotic affections. In one group they class such of the fungi as form adherent colonies consisting of long branching threads which, in older cultures, divide into spores (neither these authors seem however to be perfectly clear as to the aerial hyphæ); in another group are classed those forms in which the preponderant feature in the cultures is the short, often rod-shaped organism, like the *Wolff-Israel* fungus; and, finally, they set up a third group, the most important representative of which is a *Gram*-negative »Actino bacillus« discovered by themselves. In the classification they pay regard, besides, to certain physiological properties which seem consistently to follow the representatives of these individual groups.

In 1905 there appears an extensive and important work by *Wright*, who has set himself the task to attempt solving the old problem of the cause of actinomycosis. In by far the majority of cases he found a fungus which resembled that of *Wolff-Israel*, morphologically as well as physiologically, and, by moulding in the colonies and cutting them by means of the microtome, he demonstrated most beautifully that *the rod-shaped organisms which*

are seen in the usual smear and suspension preparations are in reality lodged in the colonies as radiating branching filaments which spontaneously divide into rods that lose coherence with one another in the process of preparation.

In regard to the morphology of the ray fungi, this is an important demonstration, even though similar observations had been made earlier, for instance by *Eppinger.*

This large and elaborate work of *Wright's,* which contains many excellent observations in regard to these anaërobic fungi, and which gives in many respects a clear, although often rather cutting criticism of previous works, by far overshoots the mark in its judgement on *Lignières & Spitz's* work, and also in its deliberations in regard to the Madura fungus discovered by *Vincent.*

Harbitz & Grøndahl (1910) arrived at similar results in their large work on human actinomycosis. They obtained very beautiful pictures of the true structure of the colonies by making frozen sections of same.

Furthermore, *Shiota* found that the pathogenic fungi in actinomycosis could be of a widely different nature. In most cases he found fungi whose morphology corresponded to *Wright's* findings, but occasionally he found in his cultures growth of microbes which distinguished themselves essentially from the former, among other things, by forming aërial hyphæ.

Pinoy (1913) undertook a grouping resembling that of *Lignières & Spitz.*

In *Schlegel's* work (1914) any attempt at classification is foregone, *Bostrøm's, Wolff-Israel's* and other ray fungi being mentioned under one.

Dresel (1914), who found morphologically highly differing fungi as the causes of clinical actinomycosis, vigorously emphasized the necessity of continuing to cultivate the organisms from these affections in order to elucidate the botanical relationships of the organisms causing the disease, and moreover points out that we have not as yet come to any clear results in regard to the morphology of the various fungi.

He does however not supply any further contribution to the botany of the ray fungi.

Besides these works, which mainly deal with the study of the actinomycosis-producing fungi, there are numerous others which describe newly discovered ray fungi, partly cultivated from various affections, partly saprophytic forms.

Of any greater interest for the botanical side of the question concerning ray fungi, are the works for instance of *Gruber, Casabo, Levy, Nakanishi, Foulerton, Silberschmidt, Feistmantel, Vallée, Gilbert, Neukirch, Schabad, Koch & Stützer, Abramow, Gjorgjevic, Claypole, Musgrave & Clegg, Plaut,* and *Rodella,* the which it will however be more profitable to take up for discussion at a later stage of the present work, just as the other works, briefly referred to above, will again be subjected to more elaborate discourse.

Likewise, I shall confine myself to mentioning the names of some of the authors who have occupied themselves with the study of the »branching« bacteria, namely: *Ernst, Babes, Nocard & Roux, Klein, Metchnikoff, Fischel, Kurth, Brun, Coppen Jones, Lubinski, Marpmann, Czaplewski, Lubarsch, Schulze, Korn, Moeller, Barannikow, Cache, Nakanishi, Spirig, Levy, Mafucci, Abbott & Gildersleeve, Kedrowski, Fontes, Much, Vilh. Jensen, Sanfelice, Vaudremer,* and many others; all these works will be referred to below.

By far the majority of all the named investigators are medical bacteriologists, the question of the ray fungi having apparently not interested the botanists to any great extent. Not until 1921 do we get a work by the botanists *Lieske'* which, in return, is planned on a very extensive scale, treating the question of the ray fungi under all aspects, including the medical one.

Notwithstanding the many excellent investigations and the enormous amount of work involved in this treatise, one gets disappointed if one expects to see the morphological problems solved. In the following we shall have ample opportunity to occupy ourselves more extensively also with this work.

In conclusion, just to state that the very newest publications suffer from the same flaws as the older ones, I shall draw the attention to *Langeron's* papers on »Mycétomes« and »Les Oosporoses« in Maladies infectieuses vol. IV. 1922, and to *Gotschlich & Schürmann's* Leitfaden der Mikroparasitologie und Serologie, 1921. In the latter, for instance, *Bostrøm's* and *Wolff & Israel's* fungi are treated under one, and, although a fine illustration of an Actinomyces bovis very distinctly exhibits an aërial mycelium, the text has no mention at all of the occurrence of such in certain ray fungi.

THE AUTHOR'S INVESTIGATIONS

As it has already been intimated in the introductory chapter, it is my intension in the following to give a morphological description of the representatives of various, in many respects widely differing, fungi; not least in respect of morphology do they differ, the only external distinguishing feature common to them all being that they are able to form genuine side-branches.*)

As far as some of the examined microorganisms are concerned — more especially those which are now usually classed with the true bacteria — the growth of these side-branches is often very sparse. Taking, for instance, the diphtheria bacillus, we may find strains which exhibit only very few true branches, so that if one has not personnally encountered such strains in which side branching is a common feature in the cultures, or in which indeed nearly all the elements are branched, it will naturally be difficult to understand the great importance which various authors attach to these branchings, when the question is of the natural systematic position of these microorganisms. For it is a well known fact that many bacterial species may occasionally display branching forms. I shall just refer to subcultures from older cultures of typhoid, paratyphoid, and dysentery bacilli, in which it will frequently be seen that the forms which first apear on the fresh medium are large, irregular of shape, and not uncommonly supplied with side-branches, while these forms will, as a rule, totally disappear after some time's cultivation; and, similar branching forms have been

*) By a genuine side-branch is meant, simply, a branch protruding laterally from a rod or thread. It is equally genuine even through a dividing line occurs early at its base, separating it from the primary rod or thread.

detected in almost every bacterial species, for instance by *Skschivan*, in the plague bacillus, and by *Reichenback* in spirillum rubrum, so that if one makes this isolated morphological feature decisive for the classification of the microorganisms in question, only very few true bacteria remain, in the classical sense of the term.

Considering however the groups of bacteria designated by Lehmann & Neumann respectively as Corynebacteria, and Mycobacteria, it will be found that among the representatives of the various species some strains will exhibit a very abundant growth of such branchings.

The bacteriologists who first discovered these especially richly branching strains, drew our attention to these forms, which, among the commonly known pathogenic microbes, are only found within the two above-named groups of bacteria.

In attempting to take up a standpoint in regard to the significance of these branching forms for the classification of these microbes within the botanical system, it will be necessary first to realize what is to be understood by relationship when the question is of bacteria and other morphologically slightly differentiated fungi.

The ideal classification would no doubt be a genealogical one. It is however extremely seldom that we can obtain any definite knowledge as to the genealogy of a microorganism; this is only the case if we have been able to directly follow the derivation of one specific form from another. All individuals derived from one cell are interrelated, however different they may be.

Likewise, two microorganisms of which we know nothing beforehand as to their origin, but which are identical in all respects, are reckoned as genealogically interrelated, inasmuch as we take for granted that they must be derived from a common ancestral stock. (Strictly speaking, we cannot be perfectly sure of being correct on this point, since it might be conceivable that two originally diverging forms have approached one another under the influence of external conditions).

Practically, this means that two fungi are reckoned to be interrelated if they respond with uniform reactions in all the tests we apply to fungi of the kind at issue, and the more heterogenous tests we posess in our technique and in which we find identical reactions on the part of the microorganisms under observation, the greater becomes the probability of their common origin.

Furthermore, we consider two bacterial strains as being genealogically interrelated, if they show such divergencies only, as we know, empirically, can arise; for instance, if the question is of two strains of bacillus anthrax, of which the one has the power of forming spores, while this power is absent in the other, we draw the inference that the one is derived from the other, reckoning the sporogenic strain as the progenitor of the asporogenic, as we know from experience that such transformation from sporogenic to asporogenic types may occur. As soon as greater divergencies arise, we possess however not even an approximately safe foundation for our suppositions as to *a true genealogical relationship.* A closer study of the development of the different variation forms may possibly in the future supply us with information as to true interrelationships of which we know nothing definite at present.

However, the term relationship is also applied in another and *wider sense of the word,* for the practical purpose of *classifying microorganisms according to essential common characters.* Here we encounter the difficulty of having to decide what characters are the more essential ones. Usually those properties which are most constant of occurrence are considered most essential, and on the basis of a sum of such constant characters in the bacteria do we divide them into groups, first the large principal groups, according to morphology, and next, subgroups, according to various reactions in tests of a highly differing nature; and, the greater the number of tests in which the bacteria show identical reactions, the nearer to one another do we place them within the system, imagining a sort of mutual relationship between the various representatives within the individual group, such as for instance the Colon-Typhoid group; this is, among other things, what is implied in the term »the natural system«. But, it is not until we have established with full certainty, the derivation of one form from another within the group, that we are absolutely justified in speaking of a true relationship; the presence of intermediate forms affords no conclusive proof that such transitions from one form to another should have taken place, or can take place.

It is this grouping according to common reactions and characters, which forms the basis of our systematic classification.

As for the value of morphology as a means of classification we find that correspondence in external form may be associated with the most profound disagreement in every other respect. So that

the purely morphological characters can only afford the basis of a very rough group division of the bacteria.

If however we consider the higher differentiated fungi, it proves feasible to undertake an applicable classification on a purely morphological basis, such as it is also usually done.

Similar considerations as those obtaining for bacteria, will of course also apply in case of the fungi, and here as there, the occurrence of intermediate forms says nothing definite as to a true relationship. Not until we have established that one form is directly derived from another, are we justified in talking of true genealogical relationship. The study of the development of such variation forms is however of the greatest interest for the elucidation of the interrelationship between the fungi, by means of which we may perhaps in the future obtain a more satisfactory systematic classification than the one we have at present to be content with.

The various groups of fungi, with which we are going to deal in the following, have, on the one hand, representatives that are rather highly differentiated, morphologically, and, on the other hand, we find among them such as are at present usually classed with the true bacteria.

The grouping of these fungi is undertaken on the basis of their morphology.

GROUP I.

The reprecentatives of this group are rather highly differentiated morphologically.

Briefly stated, the course of their deveploment is the following: A spore germinates into a unicellular mycelium, which, when exposed to free air, forms aerial hyphæ that again divide into spores which, under suitable conditions of growth run through the same course.

There being fine correspondence between the individual members of this group, we shall begin by closely tracing the development of one individual fungus under varying cultural conditions.

Actinimyces hominis Landsteiner (Kràl).

This fungus is in every respect a typical representative of the group.*)

*) As for the nomenclature, we shall leave that question open for treatment in a later chapter, making the fungi appear in this group classification under the names by which they were denoted when I got them in hand. (In a paranthesis following the name, their place of origin is stated).

By inoculating an agar plate from a somewhat older culture, which is covered with the layer of white »powder« frequently referred to in a preceding chapter, we find, by direct observation of the agar plate, minute, oval or round, highly refractive bodies, partly isolated, partly aggregated in chains or heaps. Selecting now some of the elements that are lying isolated, and following their further development, it will be seen that after some hours' incubation, they will begin to germinate, that is to say, one or several minute threads sprout from the small spherical bodies, which, simultaneously, slightly increase in size. The newly-formed filaments are always slightly thinner at their tips, than at their base, where the transition is fairly even between the body and the spore, the latter being for a long time easily recognizable on account of its larger diameter in proportion to the newly formed threads.

As said, the number of threads sprouting from the spore may differ. These threads very soon begin to form small side-branches, usually slightly thinner than the main stem and slightly tapering towards the peripheral end, these branches forming again under continued growth new lateral twigs, which initially are set very nearly at right angles to the stems.

The small newly formed mycelium is, at this stage, unicellular and with homogenous protoplasm. throughout, presenting a picture corresponding to Figures 2 and 3.

Fig. 2.
Act. hominis Landsteiner. cultivated on 3% glycerin broth agar for 16 hours at 37°. Dry lenses. Magnification about 700 times.

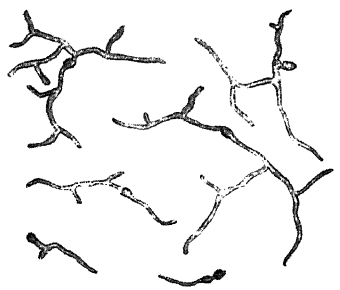


Fig. 3.
Act. hominis Landsteiner. 3% glycerine broth agar. 16 hours at 37° Oil immersion lenses. Magnification about 1000 times.

Growth continues in the same way and we soon have a small colony (for convenience sake we use the term colony not-

withstanding the mycelium being undivided), which, observed under low power, presents itself as an aureola of extremely delicate filaments radiating from the dense centre towards the periphery, with increasing distances between the individual threads.

In case the colony is placed well isolated on the medium, it may grow to a rather considerable size. Macroscopically it appears, at the outset, as a small colourless circular elevation on the agar surface, frequently with a moist sheen. Concurrently with growing along the surface of the agar, the fungus shoots its threads down into the depth of the medium, which is readily observable in a transverse section of the colony. Under continued growth, the colony preserves its circular shape, while the surface generally assumes a roughened appearance, especially round the centre, which forms the most elevated part of the colony. See Fig. 4. In older

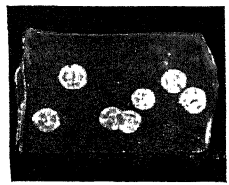


Fig. 4.
One month old colonies
Life size. Cultivated on agar at room temperature.

colonies peculiar disruptions frequently occur near the centre, imparting to the entire colony a volcano-like appearance, with a depression suggestive of a crater in the middle and evenly sloping sides. Another characteristic feature in the colonies is the small ring-shaped depression, which is constantly found along the periphery of the colonies, and which is probably due, partly to consumption of material on the part of the fungus, partly to traction exerted on the superficial part of the colony by the mycelial filaments in the depth of the medium. Other factors, besides, may play in here.

As previously mentioned, young colonies often exhibit a moist appearance and may, viewed superficially, very much resemble bacterial colonies. On trying however to remove some of the substance of the colony by means of an inoculation needle, one is surprised at finding the colony *hard and firm as cartilage*, so that

it cannot be subdivided except by means of more powerful instruments.

If we examine an older colony on broth agar, microscopically, only the most peripherally located areas prove to be accessible to direct examination with high-power dry lenses. The more or less twisted branching filaments show here, just as in the young colonies, a total absence of septa. The centre of the colonies is not accessible to direct observation because the threads are here lying in numerous layers.

Where the colonies lie closer together on the agar it is easier to study also the central portion of the colonies, because they will here be smaller and less dense of growth.*)

If this examination of the smaller colonies likewise fails to give us a distinct general view of the structure of the mycelium throughout the colony, we may attempt to cultivate the fungus on a medium which is less rich in nutritive substance, in consequence of which the colonies become less developed in size and less dense of growth, so that we are enabled to follow their development through all the phases. (This will be referred to again later).

As has been stated above, the mycelium formed no septa, by which are meant transverse lines dividing a thread into several segments. On the other hand, it will be seen that, after the lapse of some time, a differentiation of the content of the threads will occur, initiating centrally in the colonies within the older portions of the mycelium. This differentiation manifests itself by a changing power of refraction in different parts of the threads; while these at the outset, viewed with oil immersion lenses,*) appear homo-

*) In order to be able to examine the morphology more thoroughly and minutely, it will often be necessary to work with oil immersion lenses, for which purpose a cover-slip is placed on the top of the preparation. If the colonies have grown to such a size as to prevent the cover-slip from adhering everywhere to the agar, so that air-filled spaces intervene and disturb the observation, this difficulty may be overcome by depositing a drop of fluid on the agar surface prior to placing the cover-slip. The fluid will fill up the spaces between the colonies, and examination is rendered easy.

*) The difference in refraction which appears when the examination is undertaken with high-power dry lenses, one should be extremely wary of estimating. For it may be due to many different factors. If, for instance, a thread under observation is highly twisted, it will of course appear as consisting of an alternation of more and less refractive areas, according to the adjustment of the lenses. To a less skilled

genous, if perhaps slightly more refractive at the tips, doubtless due to a higher concentration of protoplasm within these areas where the rate of growth is most intense. (Similar highly refractive tips I have also encountered in many various molds, when growing on solid media).

The cause of the difference in light refraction, is that the protoplasm of the threads partly disappears at different places, while other areas of the filaments retain a similar refractive power to that originally possessed by the homogenous threads; the less refractive areas will therefore produce an »empty« impression.

The concentrated portions of the threads behave, in respect of staining susceptibility, just as the protoplasm in the homogenous threads, absorbing the stain intensely, while the intervals between them only stain weakly. This feature is most readily observed by depositing a droplet of staining solution, for instance highly diluted carbol-fuchsin, at the margin of the cover-slip, under which it is then sucked in; it will then be seen how the concentrated areas become successively more and more intensely stained. The areas which absorb the stain are of highly varying form, most frequently, however, like larger os smaller spherical grains, occasionally somewhat more irregular of shape.

To begin with, these grains are usually rather large, filling out the threads in which they are formed, but by and by they break up into smaller granules, leaving the threads more and more »empty« of appearance at long stretches.

The point of time of occurrence of these grains, and their number, vary greatly even under apparently identical external conditions; they will however constantly be found in old cultures in abundant numbers.

This »breaking up« of the protoplasm is occasionally followed by dissolution on the part if the membranes of the threads at shorter or longer stretches. The part of the mycelium that is located peripherally to this interruption, may however very well continue its growth.

observer, it may perhaps resemble a thread with differentiated protoplasm, while, if examined with oil immersion lenses, it will display a perfectly homogenous protoplasm. *Therefore, the pictures appearing in the examinations with high-power dry lenses, and with oil immersion lenses, respectively, should always be compared, when possible.*

We have here a sort of local disintegration of the fungus which, persumably, does not play any essential rôle for the reproduction of the fungus.

These granules have played a very great part in the morphological descriptions of the ray fungi, and while some authors have correctly interpreted them as grains occurring by the breaking up of the protoplasm, others have conceived them to be a sort of spores. We shall defer further mention of these granules until we have followed the development of the fungus further.

In our observation above, we had arrived at a colony consisting of a unicellular mycelium. Now, following the further development of this mycelium we shall see that, after varying intervals, there will appear some peculiar fine white specks on the surface of the colony, now in the centre, now towards the periphery, and these specks will increase in extent during the following period og growth.

Under low power these areas are seen to consist of *aerial hyphæ* which, by transmitted light, appear as almost jet black branching filaments, looking considerably thicker than the threads of the mycelium situated on the surface or in the depth of the agar. The first developed mycelium we shall call in the following *the substratum mycelium* in distinction from the later in the air protruding *aerial mycelium.*

That the threads are, as a matter of fact, projecting upwards into the air, can be readly ascertained by blowing gently on the preparation simultaneously with watching it: the aerial hyphæ will then be seen to move.

This aerial mycelium is so specifically characteristic an appearance, when examined by transmitted light, that, having seen it once, one will immediately be clear upon the nature of even the minutest aerial branch encountered in the future.

Examined with high-power dry lenses, the aerial mycelium is seen to consist of relatively thick filaments, usually forming lateral branches at an early stage.

The threads are almost uniform in thickness everywhere, looking considerably thicker than those of the substratum mycelium. The tips of the aerial hyphæ are bluntly rounded. See Fig. 5. Examining with oil immersion lenses, we are surprised to discover that the aerial mycelium is in reality considerably thinner than it appeared in the dry lens examination; the aerial hyphæ are

Fig. 5.
Act. homin. Landst. 4 days' culture.
Agar (very thin) 15°. High power dry lenses. Magnification x 700.

however usully of double the thickness as that of the threads of the substratum mycelium.

The aerial hyphæ may have a straight course, but very often they are more or less twisted in a spiral; and, the profuser the growth of the aerial mycelium, the more conspicuous become these highly twisted and winding threads, which may sometimes form quite dense tangles, almost suggestive of a ball of wool, and often attaining quite a considerable size.

Under the process of growing the aerial mycelium is frequently seen to develop some peculiar globules, on the tips or sides of the hyphæ. These globules, which in reality are droplets of fluid, lend to the microscophic picture a very characteristic appearance. See Fig. 6.

The true nature of these globules is first realized when one adds a drop of fluid to the preparation and examines under oil immersion lenses. In these cases, where the objects under observation are colonies with an aerial mycelium, it has proved most practical to add a drop of alcohol, since the aerial mycelium, which partly shuns water, will by this means be drawn down, while the morphology of the individual elements is otherwise not in the least interfered with.

Examining a preparation prepared in this way, the mentioned globules will be seen to have totally disappeared. They are thus a liquid substance, exuded by the threads, similar to what we find in many of the different molds.

The number and size of these droplets may vary considerably in the different media. Generally they occur most abundantly, and

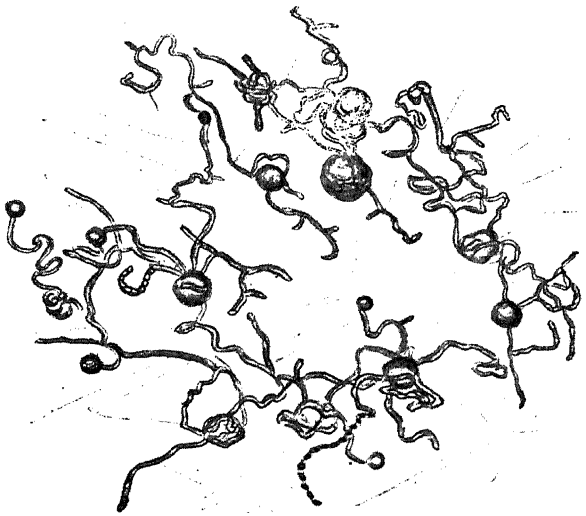


Fig. 6.
Act. homin. Landsteiner. 3 days' culture on thin agar at 37°.
High-power dry lenses. Magnification × 700.
Substratum mycelium only sketched.*)

become largest, when there is a rich growth of aerial mycelium. Another factor that plays in here is evidently the degree of moisture of the medium, since they appear in greatest abundance on a moist medium. I have not made any attempt at elucidating the nature of the content of these droplets. That there can be no question of passively deposited »exogenous« dew drops, can be easily ascertained, for instance by cultivating the fungi on a dry medium — by which no drops appear — and then place the cultures in a room saturated with live steam. By this means no droplets are formed. Likewise, it is evident that it cannot, either, be a passive process on the part of the fungi which causes the droplets to arise, since it is very usual to find two colonies placed side by side on the same medium, of which the one has numerous droplets, the other none at all. In old cultures these droplets disappear.

*) The illustrations have been but partly drawn by means of *Abbe's* drawing apparatus, because it is extremely difficult to perceive the minutest details distinctly. The outlines of the grosser elements, such as for instance the globules in this picture, are drawn by means of the apparatus, the other elements being drawn as far as possible in the right proportions.

Now, following again the growth of the aerial mycelium, we shall observe, after the lapse of some time, that the hyphæ in certain places become more irregular in outlines, so that the threads, beginning from the tips, display a series of baggings and constrictions; one might be tempted to compare the picture to a string of sausages, the constriction between the individual sausages being but slightly pronounced. Tracing this irregular thick portion of the hypha downwards from the tip, it will be seen that it passes into a regular, usually somewhat thinner filament. The next stage of development is, that the irregular portions of the filaments begin, *almost simultaneously, from the tips downwards, to divide into a series of equally sized parts of an oval or spherical shape,* lying quite close to one another; the same picture appears when examining with immersion lenses: longer or shorter chains of *equally sized spherical bodies,* lying quite *close to one another and being highly and homogenously refractive,* showing far greater refractive power than the substratum mycelium ever does. *These bodies are the spores of the fungus.* (The nature of these small bodies will be further dealt with below).

According to what has been described above, one would perhaps feel most inclined to believe that these spores were formed in the way that the mentioned constrictions were forerunners of later developed partition walls, dividing the filaments transversely into segments, the which conception will also invariably enforce itself upon one's mind until such fungi have been encountered (we find them especially among the representatives of fungi with cylindrical spores) in which the individual spores occur more scattered in the aerial hyphae.

For it is here distinctly visible that the spores are not separated from one another by the formation of septa, *but the sporulation occurs by division of the protoplasm into almost equally sized parts, without the walls of the hyphæ having any part in the process. Thus, the individual spores are separated from one another by »empty« spaces.* See graphic representation: Fig. 7.

Now, returning to Act. hominis Landsteiner, which forms spherical spores, we shall likewise here find filamentous growths with similar empty spaces separating the spores. Our attempts to demonstrate septa will be futile, whether the preparations examined be stained or unstained. Thus, in this case too, the sporulation occurs by division of the protoplasm within an aerial hypha

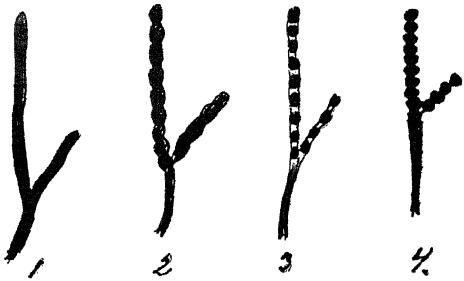


Fig. 7.
Graphic representation of aerial sporulation

into almost equally sized parts without any visible participation in the process by the filamentous membrane. Not until the division has taken place do the side walls of the hypha further contract in between the individual spores, so as to give these the appearance of a chain of streptococci.

As will appear from the above, it is not so very easy to find out in each individual case how the sporulation occurs, and the question as to the mode of formation of the spores has also been one of standing controversy.

It is far from being all aerial hyphæ which divide into spores, and most frequently only the peripheral ends of the threads are involved in the process of sporulation, the portion of the filament proximal to the medium being usually left undivided. The remaining aerial hypæ — whose part it is, presumably, to support the sporogenic hyphæ — undergo in the course of development similar alterations to those observed in the substratum mycelium, their protoplasm being subject to a similar disintegration, first into larger grains, and later into finer and finer granules, often interrupted by long »empty« spaces of filament.

The point of time for the formation of aerial hyphæ and sporulation, is widely dependent upon external conditions. If, for instance, the preparation under observation is an agar culture on a thick medium, in which the individual colonies lie scattered, and more especially if the culture is allowed to stand at room temperature where the dessication of the medium occurs at a slow rate, we may have to wait months before we see the beginning formation of an aerial mycelium, at a stage when, apparently, all growth on the part of the substratum mycelium has long ago subsided;

the same phenomenon is observed in all »good« media, that is to say such as are rich in nutritive substances. If the surface of such a rich nutritive medium is heavily inoculated, aerial hyphæ and spores will usually develop at a much earlier stage, but even in that case, months may elapse before the first sprouts of aerial hyphæ exhibit themselves.

As already mentioned, an exact direct examination is rendered unfeasible as soon as the substratum mycelium reaches a certain density of growth. This difficulty may however be overcome, either by disseminating the spores on a very thin layer of a rich nutritive medium, which will check the growth of the substratum mycelium and incite the aerial hyphæ to form at an earlier stage, or by sowing the spores on a medium which is poor in nutritive value, for instance a 2 % common filtered water-agar with or without addition of larger or smaller quantities of various nutritive substances.

The morphological features observed in these »poor« media are perfectly in accordance with those we meet in »rich« media, only the course of development has a much more rapid rate, the colonies become smaller and are much more readily observed, step by step, in their development. And, while the formation of spores, as far as many fungi are concerned, as mentioned does not set in until after the lapse of several months, and frequently very sparsely too, upon ordinary broth agar, all the fungi belonging to the group here dealt with will form aerial hyphæ after a few days' growth on water agar witout any addition at all of nutritive substances, or with the addition only of a slight quantity of glucose.

These facts are of course of the greatest importance when the question is of an exact morphological knowledge, apart from the circumstance that they may be greatly profited of for facilitating the work. (Also for the investigation of bacterial colonies the application of poor nutritive media may often be a great help).

From the above it will be easily understood that the colonies of a ray fungus, after cultivation for the same period but on different media, may exhibit a highly different appearance, so different, indeed, that it will be difficult to believe that the colonies are derived from the same fungus, unless one knows the whole facts of the case. Fig. 4, for instance, shows one month old colonies of a ray fungus cultivated on common broth agar. Fig. 8 shows the same fungus after the same period on the same medium, but with

a more liberal dissemination of spores. (Picture not good, but lack of resamblance to first named picture is sufficiently conspicuous). Finally, Fig. 9 shows the same fungus sown in a streak on a 2 % water agar after 3 days' growth at room temperature. The white specks are the aerial mycelium. The substratum mycelium is here so slightly developed as to be invisible in the picture.

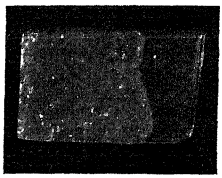


Fig 8.

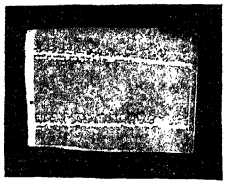


Fig 9.

Figures 10—13 may serve as examples of the differences that the aerial mycelium may present.

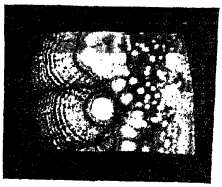


Fig. 10.
Nocardia Dassonvillei.
Broth agar. 3 months' growth at room tp.

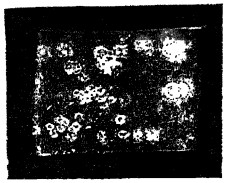


Fig. 11.
Actiomyces Vincent I
Madurae 1% Glycerine agar.
After 6 days' growth at room tp.

Fig. 13.
Act. Madurae Vincent II. Broth agar. 6 weeks
Room tp.

It is evident that the fact the aerial mycelia of these fungi are formed in very varying abundance and after widely differing lapses of time in accordance as they grow on one or another

medium, has contributed to the confusion prevailing as to the understanding of the morphology of the ray fungi. For instance, one investigator, who examines the growth (which appears to be excellent!) on a rich nutritive medium, will perhaps never encounter anything in the way of spores, unless he is a man of extreme patience, while another observer, examining the same fungus on a poor medium, will find aerial mycelium and spore formation at an early stage of cultivation; and, when we bear in mind, further, that the different fungi vary very much in their power of forming aerial mycelium at all, it does not seem so strange that we meet disagreeing morphological descriptions.

To afford a direct evidence that the nutritive value of the medium is an extremely important factor to determine the point of incidence of sporulation, I made the experiment of sowing a ray fungus on water agar, to which was added increasing amounts of glucose, starting with 1/4 %, rising to 1/2, 3/4, 1, 2, and 3 % glucose. Simultaneously, the fungus was sown on water agar, without any addition of glucose.

While a profuse aerial mycelium had formed after 2 days' growth on water agar without glucose, absolutely no aerial hyphæ could be detected on the glucose-containing media. After 3 days, there appeared abundant aerial mycelium on the 1/4 % and 1/2 % glucose agar, distincly less abundant however on the agar of the highest glucose concentration. In the 1 % and 2 % glucose agar, there ere only quite a few aerial hyphæ after 3 days' cultivation, in the 3 % glucose agar absolutely none; in the latter medium aerial mycelium did not develop until after 8 days' growth. The final result showed by far the most abundant growth both of substratum and aerial mycelia on the medium of the highest glucose concentration.

I think this experiment suffices to show that the development has a much more rapid rate of course on the poor medium owing to its lack of nutritive substances, while, on the richer media, the fungus is enabled to grow to a greater extent, while the course of development otherwise remains the same. (Many other factors doubtless also play a part here, and the total absence of aerial mycelium that is occasionally seen in the rich nutritive media, is as yet an unexplained phenomenon). At the same time, we witness how finely these fungi may accomodate themselves to new environmental conditions, and, as it were, take all the profit they can of the given circumstances.

The investigations into the forms of growth of the ray fungi on solid media were undertaken partly with water agar without enriching substances, partly with the addition of glucose in varying quantities, and with glycerine agar, bouillon-peptone-agar, ascitic agar and agar to which were added 30 % of Besredka's egg-medium.

In the broad features, the morphology is identical on all these media, apart from above mentioned variations in number and incidence of the aerial hyphæ, the cycle of development, from aerial spore to aerial spore, being the same on all the media.

The named protoplasmic granulation of the substratum mycelium may occur at an earlier stage and more abundantly in some media than in others, just as the aerial hyphæ may have a more straight course in one medium, while in another they may be more spirally twisted.

In some media the mentioned droplets are formed in great numbers in the cultures, growing sometimes in size in the course of a few days so as to become visible to the naked eye, lending to the colonies, especially on water agar, a splendid appearance suggestive of radiant brilliants.

The macroscopic arrangement of the aerial hyphæ may also vary greatly; sometimes the hyphæ are formed first in the centre, sometimes first in the periphery, where they are not uncommonly arranged in one or several rings at varying relative distances. See Figures 10—13.

In none of the media is the substratum mycelium subject to any segmentation or sporulation similar to what was seen in the aerial hyphæ. The only interruption that may occur in the course of development of the substratum mycelium is, as already mentioned, an occasional simple local disintegration of a filament.

There is not much polymorphism about this fungus, the aerial mycelium, as well as the substratum mycelium, usually displaying rather regular filaments without bulbous or club-shaped areas. Of course, among so great a number of examinations as those dealt with at present, there will always be met some divergent forms, for instance, some individual spores may be larger in size than normally in some of the cultures, but, broadly speaking, the picture is monomorphous.

It is difficult to obtain a clear impression of the appearance of the filaments located in the depth of the medium; in preparations obtained by cutting out thin discs of the medium, they do not,

however, differ from the filaments of the substratum mycelium, and do not present septa.

If we cultivate the fungus below the surface of the agar, it will be found to show the better growth near the surface, the colonies assuming here a spherical form with threads radiating in all directions. When these threads reach the surface they form aerial hyphæ.

Cultivated in a *liquid medium,* for instance broth, the picture will vary according to the mode of inoculation. In case the inoculated spores remain on the surface, they quickly germinate to a mycelium quite similar to the substratum mycelium and wwhich, after some time, forms aerial hyphæ. Eventually, the entire surface will be covered by a thick, extremely firm contiguous pellicle, resembling somewhat thick skin of boiled cream, and suggestive of those pellicles which certain molds form under similar conditions.

The aerial hyphæ have the same appearance as solid media.

If there is abundant growth on the surface of a broth test tube, the spores, which sediment in the broth, will show no development at all, or only slight development. If care be taken however, to make all the spores sink to the bottom — which may be obtained for instance by allowing them to dessicate on a piece of sterile filterpaper which is then sedimented in the broth — we shall get colonies of a very characteristic appearance, very much resembling the colonies formed by certain molds when sedimented in broth. See Fig. 14. The spores here grow to spherical flakes, the eventual size of which of course depends largely upon the density of inoculation; in case of a liberal dissemination of spores, only relatively small-sized spherical bodies appear; in case of a scattered dissemination, some of these flaky growths may attain a size which makes them fill out the test tube from wall to wall. These large, round, airy, spheres may, when fully developed, be suggestive of the ripe bloom of the dandelion. The broth keeps perfectly clear throughout growth. These spheres prove to be rather resistent to mechanical action, a rather vigorous shaking being necessary to make them break up into smaller flakes.

Their morphology is identical to that of the substratum mycelium, no septa or spores being found. We meet also here the division into granules on the part of the protoplasm, known from the substratum mycelium.

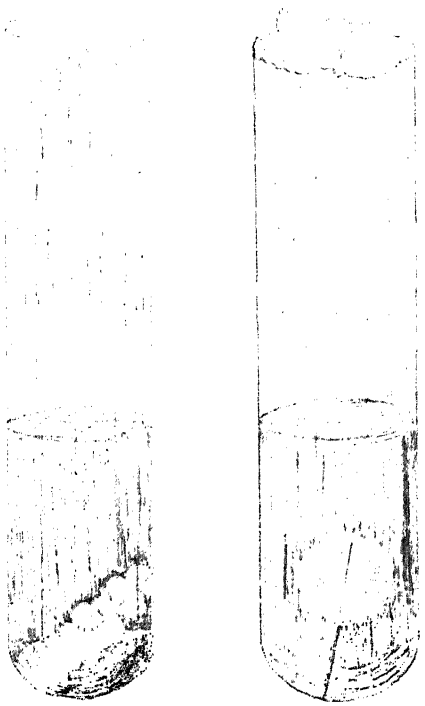


Fig. 14.
Act homin. Landsteiner. Broth culture. 3 weeks at room tp. The »spheres« are supported by the mentioned piece of filter paper. Life size

As growth progresses, the spheres may fill up the broth from bottom to surface, and may here form aerial hyphæ and spores, *usually however not until after many months' growth* (of course depending also on the amount of broth). This is a feature which seems not to have been generally known. *Mertens*, for instance, found, in regard to one of the strains with which he worked, that, at the outset it formed small spheres and then larger flakes at the bottom of the broth tubes, to form eventually, a surface growth with a white layer of spores at the end of 5 months' cultivation. This is however a quite normal course of development, and it is doubtless a wrong inference *Mertens* draws from these findings, inasmuch as he concludes that a transformation

should have taken place from the anaërobic *Wolf-Israel's* type of ray fungus to the aërobic *Bostroem* fungus.

In gelatine, which is liquefied by the fungus, it has a similar growth as in broth.

Attempts to produce morphologically atypical forms by cultivation on media to which were added substances which, empirically, produce such in bacteria, gave no positive results. The fungus fails to grow on $^1/_2$ % caffein agar, and, at lower concentrations it showed no morphological alterations. Cultivation on 3 % LiCl agar, which gave abundant growth, did not produce any forms that were morphologically atypical.

The morphological characteristics of this fungus are therefore, briefly stated, the following: The spores grow to a unicellular, branching mycelium, which affords the substratum for an aerial mycelium consisting of somewhat thicker, branched filaments, which form spores by a simultaneous division of the protoplasm in the threads, progressing from the tips towards the base.

In broth the fungus grows as an undivided mycelium, eventually assuming the forms of airy spheres which, when reaching the surface of the broth *after long time's growth,* may form aerial hyphæ and spores.

The fungi dealt with in the following section are morphologically closely allied to Actinomyces hominis Landsteiner; the various representatives present of course certain individual differences, which however, apart from the shape of the spores, are of minor importance and of such a varying nature within the individual species — just as it was seen in the case of Act. homin. Landsteiner — that an exact account of these minor morphological divergencies would be of practically no value for future investigators.

For, while it is possible personally to learn to distinguish between the fungi one works with by means of such minor recognition marks as, for instance, especially twisted aerial hyphæ, especially arched colonies, or especially beautifully defined annular formations of the aerial mycelium, it is extremely difficult to impart one's experiences to others owing to above mentioned variability of the differences. Among these varying characters are the colour of the aerial mycelium and also the different colours displayed by the substratum mycelium, sometimes sharply defined to the mycelium, sometimes diffusing into the culture medium. As far as most fungi

are concerned, these colours may vary within a very wide range.

One fungus may, for instance, display distinctly green aerial hyphæ when growing on water agar, while the hyphæ become white on broth agar; and another fungus, having a violet substratum mycelium on broth agar, may be colourless with yellowish white spores on water agar, just as we may encounter ray fungi with a substratum mycelium of an ochreous yellow on broth agar, the same having colourless mycelium on water agar.

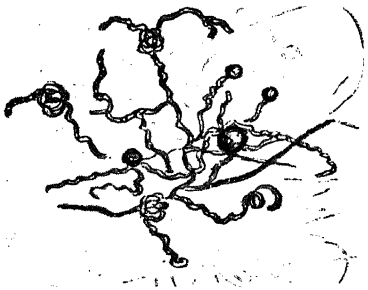


Fig. 15.
Act. Madurae Vincent. Thin agar culture, 24 hours at 37° and 3 days at 15°. Dry lens system. Magnification × 700. Substratum mycelium merely sketched.

But, while all these characters may vary, the morphology remains constant, taken in the same sense as for Act. hominis Landsteiner.

In the following I shall therefore restrict myself to mention such morphological characteristics as are either of special interest, or which we have not encountered before.

Actinomyces Madurae Vincent. (Kràl). I & II.

Of this fungus I have examined two different strains, which were identical as far as morphology goes. The first strain, originating from *Kràl's* Institute, behaved exactly as described by *Vincent*, and later by *Babes, Brault*, and others. It formed a unicellular, delicate branching mycelium which, after varying lapses of time, formed aerial hyphæ, which again divided into spores (Fig. 15). In the meantime there appeared however a work by *Lieske*, showing on this point a rather considerable divergency from earlier authors' and my own experiences. *Lieske* briefly records the fol-

lowing about the Madura fungus examined by him (Page 250): »Der Krankheitserreger des Madurafusses ist ein echter, langsam und vorwiegend aerob wachsender Strahlenpilz. Durch das Fehlen der Luftsporen und den leichten Zerfall der vegetativen Fäden erinnert er morphologisch sehr stark an die anaeroben, menschenpathogenen Strahlenpilzenformen; er nimmt eine Mittelstellung zwischen diesem und den gewöhnlichen saprophytischen Stämmen ein.«

Fig. 16.
Act. Madurae Vincent II. 3% glycerine agar. One week's culture at room tp. Immersion lenses. Magnification ×1000. (See also Figs. 11 & 14.

This description is the more strange inasmuch as it was the same strain from *Kràl's* Institute that had been examined. For, there being the greatest difference between the original description of this fungus and that of *Lieske,* and not as he himself claims in direct reference to *Vincent's* work, fine agreement with this author, I enquired at *Kràl's* Institute whether the fungi sent to *Lieske* and to myself were identical. Professor *Pribram* — to whom I am greatly indebted for genial courtesy in promptly forwarding the desired fungi — kindly replied that the Institute possessed this one strain only.

There is thus a profound difference between this fungus described by *Lieske* and the »classical« Madura fungus, however this difference may be explainable.

Later I received furthermore another newly acquired Madura fungus from *Kràl's* Institute, which in every respect showed identical relations with the one previously examined. Figure 16 gives a picture of the aerial *mycelium* in which the details of spore formation are especially distinctly seen. *Koch & Stützer* likewise

examined a Madura fungus. Their description of the leather-like, strongly adherent colonies leaves little doubt that the fungus they worked with was *Vincent's* Madura fungus.

They found no formation of spores in their cultures, an absence that is readily understandable when the investigators have not realized the conditions for the occurrence of such, and the necessity of following the growth of the culture in the usually employed media for a period exceeding the customary one when the question is of other microorganisms. It cannot of course be excluded that this strain may have lost its power of forming aerial mycelium and spores, but I feel convinced that the records of ray fungi of this group that fail to form aerial mycelium, will become less in number, if the more adequate media be employed for these investigations.

Pinoy's description of the Madura fungus is likewise in accordance with the classical one. He did find aerial hyphæ, if however scanty in number.

Nocardia Dassonvillei (?) Institut Pasteur) (supposedly the same fungus as the one mentioned by *Langeron* 1921, Page 433, cultivated by *Brocc. Rousseu* from conjunctivitis and from throat-phlegmone). This microbe shows identical relations with above-named fungi. Especially characteristic is the beautiful annular arrangement of aerial hyphæ (See Fig. 10), *the which may also be found on the surface of a liquid medium*, a feature I have not witnessed in any other ray fungus. Without entering further into the question of the conditions for the occurrence of these »rings«, I just want to draw attention to this mode of arrangement of the aerial hyphæ on the surface of broth, because it seems to indicate that a very essential factor for such »ring« formation should be to seek in the constitution of the fungus itself, as it seems to me difficult to conceive that the external conditions of growth should change 5 to 6 times in the liquid medium in the course of 2 to 3 days. (The culture was kept in a dark room so that the significance implied in change from day-light to darkness may be left out of consideration).

It is far more difficult to judge of the change of external conditions when the question is of solid culture media.

The spores formed by Nocardia Dassonvillei are round or slightly oval of shape and easily discernable with a high-power dry-lens.

Actinomyces Bostrøm (Morgenroth) is morphologically identical with the previously named fungi. It forms round spores.

Actinomyces albus Berestnew (Kràl). Round spores. Typical in every respect.

Leptothrix buccalis Winslow. (Kràl). Spores cylindrical and elongated oval. In the species of ray fungi which have cylindrical spores, the filaments do not, as in the species with round spores, form constricted areas in between the spores, so that these are not distinctly visible except when viewed under immersion lenses. In these fungi may also be seen the often rather extensive empty intervals between the individual spores, so as to leave no doubt as to the mode of formation of the spores, that is, by division of the protoplasm without any primary participation in the process on the part of the membrane. The species which form cylindrical spores usually exhibit a less twisted aerial mycelium than that of the round-spored species.

Actinomyces rosaceus (Kràl).

In most media this fungus develops an extremely abundant growth of aerial mycelium (See Fig. 17); the red colour is however usually but slightly pronounced. The spores are cylindrical.

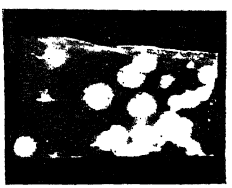


Fig. 17.
Act. rosaceus. Cultivated on agar, 14 days at room tp.

Actinomyces Lieske No. 16. (Karlsruhe).

Actinomyces Lieske No. 47 (Kràl).

Actinomyces Lieske No. 51 (Kràl).

The latter two were cultivated from swabs from tonsils. They are all of them typical representatives of this group.

Streptothrices I & II were cultivated by the author from wood-

land soil. They were typical in every respect. In water agar these fungi present an especially beautiful picture, common to the representatives of this group, the sporogenous hyphæ being often situated like a small crown of branches on a common stem, which does not participate in the spore formation. (See Fig. 18).

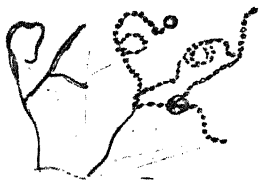


Fig. 18.
Streptothrix I. Cultivated 2 days on water agar at room tp. Dry lenses. Magnification × 700.

Actinomyces (Morgenroth), identical with the others.

Actinomyces violaceus (Landbohøjskolen i e. Institute for Agricultural and Veterinary Science, Copenhagen).

Production of a purple pigment diffusing all through the media. In some media, however, no pigment is produced, and even in the same medium this character may vary.

The fungi mentioned below were isolated from swabs taken from a batch of soldiers lying in summer camp in the country, where they had therefore ample opportunity to inhale aerial spores from various ray fungi. In no less than 40 cases out of 400 swabbings did I succeed in obtaining cultures of ray fungi, that is about 10 per cent of the aggregate cases.*)

The fungi are denoted by the department numbers of the soldiers.

*) It seems to me that this gives rise to certain suggestions when the question is of pure cultivation of microorganisms from cases of actinomycosis. For, when it proves that these ray fungi can be so commonly isolated by swabbing, one grows instinctively a little sceptical towards, for instance, *Bostroem's* statements in regard to these fungi as being the causative agents of actinomycosis, when it is taken into consideration that in certain cases he had to inoculate up to one hundred test tubes in order to obtain growth of one colony in one tube only. The growth obtained may then very well have originated from an adventitious aerial spore, having no connection at all with the disease.

Streptothrix $^1/_{20}$. I am going to deal a little more elaborately with this fungus owing to its forming some rather peculiar »capsules« when cultivated on water agar.

As soon as the aerial mycelium begins to sprout, minute spheres with a high refractive power begin to appear, scattered, on the outside of the filaments. These spheres increase in size during the following period, and after the lapse of some time, we see a picture like that of Fig. 19, showing how these spheres, gradually,

Fig. 19.
Streptothrix $^1/_{20}$. Water agar. 48 hours. Immersion lenses. Magnification × 1000.

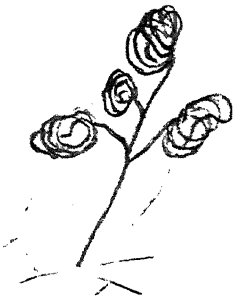


Fig 20
Streptothrix. $^2/_{219}$. Substratum mycelium sketched. Magnification × 700.

according as they grow, creep around the filaments, by which they assume the shape of coffee-beans. These bodies do not adhere very strongly to the filaments, and they preserve their shape when falling off. They are insoluble in alcohol, ether, or chloroform. They take no part whatever in the special development of the fungi, and, strange enough, they are not produced in any other medium than water ager.

(In the chapter on the growth-forms of ray fungi within the animal organism, this fungus will be further mentioned).

This is the only one of the examined ray fungi in which I have found these small bodies, except for one isolated case of Actinomyces Eppinger, in which I detected a few forms of a similar nature.

Streptothrix 1/38. Round spores.

Streptothrix 2/219 is distinguished by the formation of especially large and profusely tangled balls of aerial mycelium. See Fig. 20

Streptothrix 2/559. Cylindrical spores.
Streptothrix 1/9 555. » »
Streptothrix 4/413. Round »
Streptothrix 2/516. » »
Streptothrix 1/647. Cylindrical »
Streptothrix 2/10. Round »
Streptothrix 2/518. Round spores.
» *4/730. Cylindrical spores.*
» *2/527.* » »
» *3/41.* Round spores.
» *1/8.* » »
» *2/219².* » »
» *84.* » »
» *4/41.* » »
» *3/342.* » »
» *1/155.* » »
» *3/457.* » »
» *4/40.* » »
» *4.* » »
» *4/764.* » »
» *23.* » »
» *162.* » »
» *1/31.* » »
» *2/56.* » »
» *4/517.* Cylindrical spores.
» *1/3.* Round spores.
» *3/46.* » »
» *3/41².* » »
» *R. 4.* » »
» *R. 5.* » »
» *R. 7.* » »
» *R. 1.* » »

Morphologically all these fungi thus correspond with one another, except for the shape of their spores; nevertheless, the cultures of them present a highly different appearance on the same medium. Some form spores at an early date, and profusely. Some produce pigments, that may be of highly varying colours: green, pink, yellow, black, brown, etc., and, physiologically, too, they are different; some of them, for instance, possess a strong hæmolytic power,

while such is totally absent in others; on the whole, they present so many differences that not two of them can be said to be absolutely identical to one another.

One physiological property seems however to be common to all the fungi belonging to this group, that is, the power of liquefying gelatine. This property is however differently pronounced, and, in some cases, does not occur until after a long time's growth, a fact which perhaps explains the occasional statements by some authors that the fungi examined by them failed to liquefy gelatine. Vincent, for instance, states that the Madura fungus does not liquefy gelatine, while for instance *Babes, Koch & Stützer* and the present author find that it does. It cannot of course be precluded that both observations may be correct.

These physiological investigations have been of a merely orientating nature. All the fungi here dealt with show constancy in their morphology, and I have not succeeded in substantiating any of the transitions, — taken in the sense of transformations — mentioned by *Lieske* as being of frequent occurrence, from sporogenous to non-sporogenous, and, conversely, from sporeless to spore-producing forms. That a colony on a certain medium may appear as sporeless for several months, and occasionally quite fail to form spores, is a quite ordinary feature, but, on sowing subcultures from such sporeless colonies, I have constantly found, at any rate in water agar, that they formed spores again. Likewise, I have many times observed that there may be sectors, also within one single colony, which are mutually different in regard to spore-formation; but, on inoculation into a suitable medium from the sporeless sectors, the aerial hyphæ always reappeared. The most natural explanation of this seems to me to be that the *sector colony should have arisen from several spores*, which have begun to germinate at different points of time, so that the first germinated mycelium has exerted an inhibitory influence on the mycelium of the later germinating spore, the latter thereby losing its power to form spores under the given conditions. Such an inhibitory action on the formation of the aerial mycelium of a colony by influence from a neighbouring colony, is a quite usual finding, and, moreover, another factor that also indicates that the sector colonies are usually mixed colonies, is the fact that it is possible experimentally to produce sector colonies by mixing the spores from two species, of which the one has for instance a substratum

mycelium of an ochreous yellow, and the other a colourless mycelium, and then sow from this mixture. Some of the colonies formed in the subcultures will prove to consist of yellow and colourless sectors. There is however no reason for doubting that there may arise spontaneous changes in a colony originating from one single spore, and such are of the greatest scientific interest for the questions concerning relationship and heredity, but, as far as the ray fungi here dealt with are concerned, in regard to which external conditions have been seen to exert an enormous influence on the appearance of the colonies, one should be extremely wary in one's judgment. Moreover, it would seem appropriate to have the term »mutation«, which is so extensively used in bacteriology, replaced by another term, since the very criterion for the reality of mutation, sexual reproduction, is absent in the bacteria and lower fungi.

Now, on comparing the group of fungi here described with previous works on ray fungi, it is not difficult to find the fungi which belong here, owing to their specific characters.

An investigation of this nature soon proves that the earlier authors have performed the best work as far as morphology is concerned. *Vincent's* and *Sauvageau & Radais'* works stand out prominently, the cause of the good results obtained by these authors being that they have endeavoured to directly follow the development of the fungi, most frequently in hanging drop cultures of broth.

Sauvageau & Radais, in effect, give a description which in most features is in accordance with the above description, of the unicellular mycelium that forms the basis for the formation of aerial hyphæ, which divide into spores. The interpretation of the more subtle details of sporulation is however not quite correct.

They point out quite correctly, too, the occurrence of granular bodies of varying size within the protoplasm of the unicellular mycelium. In *S. & R.s* work we meet for the first time the term *fragmentation,* by which they characterize just this disintegration on the part of the protoplasm into pieces and granules of varying size, while they emphasize, quite correctly too, that this disintegration is a phenomenon which has nothing at all to do with true spore formation. Simultaneously, they had fully realized that other portions of the fungi than the spores were equally able to form the basis of new colonies, since they showed that parts of the

»fragmented« mycelium, prior to the incidence of sporulation, could germinate into new typical colonies of ray fungi.

Erroneously, they believed that the spore formation occurred by the formation of transverse dividing lines, emerging from the side walls of the aerial hyphæ, which should thus be divided into equally sized *segments*. They sharply emphasized that such dividing lines did not arise in the substratum mycelium.

Lachner Sandoval pointed out the same relations in the fungus examined by him, never finding any transverse division of the substratum mycelium. Otherwise, he adopts the terms given by *S. & R.*, fragmentation and segmentation, and conceives the spore formation in the same way as those authors.

But, notwithstanding these fine works which gave us an understanding of the morphology of these fungi and emphasized the great difference between ray fungi of this group and bacteria, it is but seldom that more recent authors have known to apply these earlier experiences to their findings, the essential reason of which being the inadequacy of the methods of investigation employed.

Another cause of the prevailing confusion is, that the once established terms have not been retained in their original sense. The term fragmentation is often used in the sense of segmentation, and vice versa. *Neukirch*, for instance, is wrong in claiming to be in agreement with *Lachner Sandoval* and *Sauvageau & Radais* when he writes the following in his polemics with *Gilbert:* »Nun habe ich, wiederum ebensowenig wie *E. Levy*, in die Elemente des kreidingen Belages Oidiensporen genannt sondern Fragmentationssporen.« In the latter quotation we find moreover one more designation for the spores, which other authors again name »arthrospores«.

As far as I know, *Shütze* was the first to point out precisely that the formation of spores takes place by a primary division of the content of the filaments, without any action on the part of the membrane.

The »white powder«, the »chalky layer« etc., are terms we constantly meet in the literature, and while the various authors could of course not help seeing that this »powder« consisted of small regular bodies, connected in chains, or situated singly, their interpretation of them is greatly at variance. Some conceive them as spores, others allege that they cannot be such; *Mertens*, for instance, claims that they cannot be spores because they do not

behave like bacterial spores! And, taking a quite recent work by *Rodella*, the following quotation will suffice to show how absolutely earlier experiences have been lost; the question is about a ray fungus which, according to the whole description, comes under the present group:

»Nach Ablauf von cà. 3—5 Tagen bedecken sich manche Kolonien mit einem staubigen, weissen Ueberzug der mit dem Platinspatel oder der Platinøse sehr leicht abzuwischen ist. Es handelt sich dabei auf alle Fälle nicht um eine Verunreinung, sondern nur um eine charakteristische Eigentümlichkeit, wie wir uns auf Grund von vielen Versuchen überzeugen konnten. In gefärbten mikroscopischen Präparaten zeigt der weisse Belag auf der Platte mehr oder weniger lange Fäden, die mit kleinen Kokken gefüllt waren. Bei der Ueberimpfung von der Agarplatte in Bouillon bekamen wir hier und da Kulturen welche sich trübten. Die Trübung war jedoch nicht so intensiv wie die einer gewöhnlichen Staphylokokkenkultur, sondern etwas mehr staubig. Nach kurzer Zeit klärte sich die Flüssigkeit und es bildete sich in weisser, punktförmiger Niederschlag. In dieser Kultur waren lauter kleinen, runde Kokken zu sehen, die z. T. Gram positiv, z. T. Gram negativ, entweder vereinzelt oder in Haufen angeordnet waren. Diese Kokken waren nur ungefähr halb so gross wie diejenigen des Staphylococcus pyogenes albus.«

It seems strange that *Rodella* should not have been more surprised at discovering that a ray fungus suddenly generates staphylococcus-like microorganisms which, moreover, continue development as staphylococci on the bottom of a test tube with broth. If this observation were correct, it would be of the very greatest scientific interest from the point of view of systematization of bacteria. The explanation of this finding is however, probably, that the aerial spores, which have presumably been liberally inoculated, have sedimented in the broth and remained unaltered at the bottom of the tube. For it is a fact that certain of the ray fungi belonging to this group occasionally fail to develop on the bottom of broth, especially if the surface is far above.

When *Rodella*, in the same work, is a little hard on *Brussoff*, this seems unjust, since it is to *Brussoff* that we owe one of the finest more recent descriptions of ray fungi of this group.

In the above, we have constantly mentioned the round, oval or cylindrical elements formed by the aerial hyphæ, as spores; we shall now define a little more closely with what right.

It is not spores of the same nature as bacterial endospores. They distinguish themselves from these both by the mode of their formation and by their far lower power of resistance; and, finally, they differ in tinctorial respect.

The term, spore, is however also used in a different sense, for instance, in regard to lower fungi, the spores of which bear no resemblance either to the endospores of the bacteria. Having in vain sought for a definition of the term spore in various handbooks dealing with these fungi, I shall just state what I understand by spores as far as ray fungi are concerned, namely:

Bodies that are identical to one another in form, which have a special mode of formation, and are distinguished by a greater power of resistance than the mycelial filaments, and which, under adequate conditions, grow out into a new mycelium. It is necessary to emphasize their greater resistance, for, as already referred to, fragments of the mycelium are likewise able to form a new mycelium. There is however the difference between spores and fragments of substratum mycelium, that almost all spores will germimate on a suitable medium, whereas, under identical conditions, *only a minor part of mycelial fragments are able to germinate, and the older the mycelium, the more reduced becomes the germinating power of its individual fragments.*

We shall have, here, to occupy ourselves a little further with the often mentioned spherical and granular bodies which arise in the substratum mycelium, because these have from several sides been conceived as spores and correlated with the aerial spores. Their resemblance with the latter is however quite superficial. They distinguish themselves from the aerial spores by being unequal in size, and by being less refractive. The continuous breaking up into smaller and smaller granules is in itself sufficient to preclude the idea of spores. Nor do these granules play any part whatever for the germination of the mycelial fragments, since those fragments of mycelium which have retained a homogenous protoplasm show a far more constant power of germination than those which have developed the protoplasmic granules.

This would supposedly suffice to show that the substratum mycelium does not form spores.

True spores are always perfectly homogenous of structure, and remain unchanged when once formed.

The previously mentioned experiment with cultivation of ray

fungi on media of varying nutritive content likewise indicates that it is spores which are formed at the point of time when the development is otherwise about to be discontinued. (Just the same as is the case in regard to sporulation in bacteria, where this does not set in until the vegetative development is about to stagnate).

As for the greater power of resistance of the spores, this has already been pointed out by several previous authors.

Lachner-Sandoval thus found, by examination of Streptothrix albido-flava, that the spores withstood about 15° more of heating for 3 minutes, than did the vegetative mycelium. *Lieske*, however, finds no mentionable difference in the thermal resistance of the vegetative mycelium and the spores, respectively.

On undertaking experiments of this nature, one should of course make sure of actually working with sporecontaining and sporeless cultures, respectively. The most reliable method to ascertain whether a culture has formed spores, is to examine it directly under the microscope; it is not sufficient macroscopically to detect that an aerial mycelium has been formed, for several days may occasionally elapse before this mycelium differentiates into spores.

It is however difficult to make sure that a culture is sporeless, since it sometimes happens that some indvidual spores wait to sprout for several days. Thus, if the examined culture be too young, the results may sometimes be misleading.

In my own experiments I obtained the best results by the following course of proceedings: an agar plate is inoculated with the fungi to be examined, and allowed to stand for some days. Having ascertained that no aerial hyphæ have formed, an excised cube of the agar is by means of a strong inoculation needle, bent at an angle, plunged into a test-tube with broth, and incubated for some few days. Now, one may feel sure that all the spores which are on the whole able to germinate, have done so. If the question is to examine the thermal resistance of the spores, I have endeavoured to make the experimental conditions as near identical as possible, by smearing out the spores on an agar plate, out of which cubes are then excised in the usual manner, and carefully immersed into the broth. The spores adhere well to the agar in this way, which may be ascertained by means of a series of controls, where the tubes containing such inoculated agar cubes in broth are placed in the incubator without having previously been heated. There

will then never occur primary surface growth. The heating took place simultaneously of the spore-containing and sporeless tubes in water bath, taking well care that the tubes were deeply immersed in the water, which always reached above the level of the broth.

I have found it more to the purpose to keep the experimental temperature at a somewhat lower level than *Lachner-Sandoval* and, instead, increase the period of heating. Further, in order to obtain clear results from such experiments one should select strains which grow well at the bottom of broth. After heating, the tubes are allowed to stand at room temperature, or at 37°, to be examined at the end of some days. New colonies, if such have been formed, will then exhibit the characteristic picture of loose snowballs, adhering to the agar.

These experiments showed the spore-containing material to be considerably more resistant than the vegetative mycelium.

I.

	55°	1 hour	2 hours	3 hours.
Actinomyces Lieske No. 48				
+ spores	»	+	+	+
÷ »	»	÷	÷	÷
Actin. alb. Berestnew.				
+ spores	»	+	+	+
÷ »	»	÷	÷	÷
Actin. Bostroem.				
+ spores	»	+	+	÷
÷ »	»	÷	÷	÷
Nocardia Dassonvillei				
+ spores	»	+	+	+

I need only state these few examples out of many in order to show that there is a rather considerable difference between the spores and the vegetative forms, in their thermal resistance.

These ray fungi are extremely resistent against desiccation; *Lieske*, for instance, has demonstrated that sporeless as well as spore-containing material withstood desiccation for eighteen months. This result does however not prove that the vegetative mycelium is equally resistent as the spores, against desiccation.

II.

		2 hours.	50°	55°	60°	65°
Nocardia Dassonvillei.						
	+ spores	»	+	+	+	+
	÷ »	»	÷	÷	÷	÷
Actin. Berestnew.						
	+ spores	»	+	+	+	+
	÷ »	»	÷	÷	÷	÷
Actin. Bostroem.						
	+ spores	»	+	+	+	+
	÷ »	»	÷	÷	÷	÷
Actin. Lieske No. 48.						
	+ spores	»	+	+	+	+
	÷ »	»	÷	+	÷	÷

An experiment for the purpose of deciding this question would probably have to cover a far longer period.

The experiments performed by myself did not extend beyond about 8 months and did not display any distinct difference in the degree of resistance against desiccation on the part of the mycelium and the spores, respectively. The technique applied will be readily understood by means of the present drawing, Figure 21. The bottom of the test tube is blown off by first heating it intensely in a gas flame and then cool suddenly by immersing in cold water. The rack with the test tubes is dry-sterilized and the strip of filter paper moistened thoroughly in an emulsion of spores and mycelium, after which the rack is placed in incubator at 37°. It is now quite easy, at proper intervals, to make a control test by arming a tube rack with the same number of broth tubes as that of the test, and cutting off small pieces of filter paper corresponding to the mouth of the broth tubes, and making these plunge to the bottom of the broth.

Lieske states that sunlight exerts no deleterious effect on either mycelium or spores. There must however have been some error or other in *Lieske's* experimental device, for in all the experiments performed by the present author, it was quite evident that the young mycelium of the fungi was vigorously acted upon by sunlight. The experiments were arranged so that spores were libe-

rally disseminated all along the surface of large Petri dishes containing a 1 % glucose agar. These were now placed, bottom upwards, so that the sunlight had to pass the agar layer of about 2 mm's depth before reaching the germs. A piece of opaque black paper was pasted across one half of the dishes, which were now

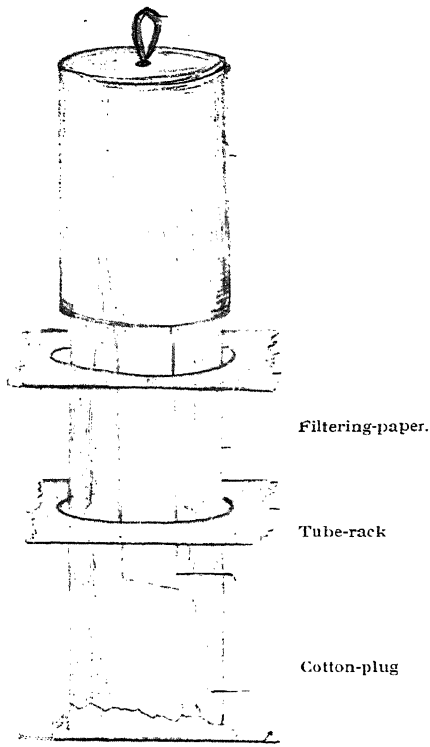


Fig. 21.

allowed to stand uninterfered with for the following days, being exposed to direct sunlight for a few hours only in the afternoon. After the lapse of some few days, the plates presented a very characteristic appearance. The half of the plate which had been exposed to the light, showed no macroscopically perceptible growth, while the other half was covered with white aerial mycelium.

On examining microscopically the part of the surface where

no growth appeared, it proved that the spores had germinated (probably in the night) so as to form a small mycelium, which had then been checked in its growth by the action of the light. Now, on placing such a plate in the dark in the purpose of finding out whether growth in the part of the plate which had been exposed to the sun, was totally abolished or only restrained, — no growth appeared, and, the medium proving to be unimpaired by a repeated inoculation of spores, it was therefore evident that the sunlight had killed the germs, the deleterious effect having affected the young mycelium and not the spores, since the latter can withstand sunlight for a very long period without impairment to their germinating power.

An example that the mycelium may be extremely susceptible to light was afforded by a plate which was kept in the shade and never exposed to direct sunlight. Its surface, being evenly inoculated with spores, gave growth only in those areas which corresponded to the shadow cast by the name of the fungus written on the Petri dish by means of a red pencil. The greater resistance to various external actions, and the specific mode of formation, distinctly proves that the small spherical elements formed by the aerial hyphæ, are spores.

Where do now these fungi belong within the botanical system? It is obvious that they form a well defined group, which shows but superficial points of resemblance with the bacteria on one side, and with the Hyphomycetes on the other.

On studying the handbooks treating on sporogenous fungi, for instance *Engler & Prantl's, Kolderup-Rosenvinge's,* and others, it will prove impossible to find any group with which they can be naturally classified, and, comparing the ray fungi with the many various molds occasionally encountered in one's cultures, no points of resemblance can be found between such and the ray fungi here dealt with. Among other things, these molds distinguish themselves by a much coarser structure, they are all of them »giants« as compared with the ray fungi, as *Lachner-Sandoval* expresses it. Many and long discussions have been carried on in the course of times as to whether the ray fungi are more closely allied to the Hyphomycetes, or whether they should be classified among the bacteria, and, while for instance *Vincent, Sauvageau & Radais,* and *Lachner-Sandoval,* — who worked exclusively with fungi belonging to the group here dealt with, — agree that these differ from

the bacteria in all essential points, several more recent authors seem inclined to classify them nearer to the bacteria. The latter point of view is no doubt due to the circumstance that these authors have worked with such branched fungi as well which display several points of resemblance to the bacteria, and, having then, anticipatively, united alle the branching fungi into one large group, the group here dealt with quite naturally followed suit.

Thus *W. Kruse* writes for instance: »Es kann kaum ein Zweifel unterworfen sein, dass die Ahnlichkeit der Streptotricheen mit den Schimmelpilzen nur eine äusserliche ist, während sie den Bakterien nahe verwandt sind. Besonders die Gruppe der Diphtherie und Tuberculose kommen hier in Betracht.« And further on *Kruse* says: »Die Ahnlichkeit wird dadurch noch grösser, dass die einzelnen Streptotricheen je nach Umständen bald das Wachstum in verzweigten Mycelien bald Vervielfältigung nach Art von Bacillen auftritt.« It has however never been demonstrated with certainty that the ray fungi are able to multiply in the same way as bacteria do. The fact that we find bacteria-resembling elements in smear preparations from certain ray fungi, gives us no information as to the situation of these elements within the colonies, which is, in effect, the essential matter as far as the question on the systematic position of the fungi is concerned. And, in regard to the fungi dealt with in this chapter, a transformation to more bacteria-like forms has never been demonstrated with certainty. The resemblance of the spores with cocci is of course so superficial that it would never occur to one to attach any importance whatever to it, when one first knows the mode of formation of the spores.

To find these views of *Kruse's* advocated by a modern botanist, *Lieske*, who has worked very intimately with these fungi, seems indeed more surprising. In his case a defective technique has presumably had the effect that apparently slight, but probably highly essential differences, have escaped his notice, and he unites all the different groups of ray fungi into one large common group. Within this group he finds all gradual transitions from the group here dealt with, to forms that are more like bacteria.

A thorough study of the group of fungi treated in this chapter will easily show that it would be extremely difficult to find one single morphological feature in common with the true bacteria. And I cannot help finding that it is a scientifically rather deficient

comparison *Lieske* makes on Page 44 (Figs. 9 & 10) when, in his argumentation in the benefit of resemblance with bacteria, he compares two pictures, of which the one represents diphtheria bacilli, and the other the cylindrical spores of a fungus belonging to the group of fungi here dealt with. It is not feasible thus to detach an element from its connection in a relatively highly differentiated fungus, and compare it with an organism, which exhibits a superficial likeness in this one point, while it fails to show a whole series of morphological characteristics. Only a reliable demonstration of a transformation from one of these ray fungi to a less differentiated microorganism which, morphologically, shows the same relations as diphtheria bacilli, would justify our talking of relationship and our attaching more importance to the points of resemblance than to the points of difference.

In conclusion, we shall mention a fungus which morphologically shows some divergency from the above mentioned fungi, but which, on the other hand, presents so many points of resemblance that it would be most practical to classify it with this group, among other things because previous investigators, *Harbitz & Grøndahl,* have found that it may form an abundant aerial mycelium. Since *Harbitz & Grøndahl's* Actin. Affanassiew was derived from Kràl's museum like mine, there is every reason to believe that the fungi in question here have been identical.

The colonies of *Actinomyces Affanassiew (Kràl)* quite resemble the colonies of this group (apart from their not forming aerial mycelium), just as the growth in broth occurs in the same manner in smaller and greater loose flakes, which may successively attain a considerable size. I have never witnessed growth on the surface of liquid nutritve media, nor have I ever seen this fungus form an aerial mycelium in any medium, as long as it has been in my possession.

Therefore, in case the fungus actually is identical with the one with which *Harbitz & Grøndahl* worked, a change must have taken place in this fungus, which apparently is constant.

The cultures are thus quite analogous with the cultures of the fungi of Group I, except for the absence of aerial hyphæ, and, on examining the large loose flakes and spheres that arise in a broth culture, we shall here, no more than in the other fungi belonging to Group I, be able to find division lines in the mycelium.

On the other hand, if we examine the growth on solid media,

a difference will appear between this fungus and the others of Group I. At the outset, the resemblance is complete also here, the mycelium consisting of very delicate slender filaments, undivided all along their course.

Gradually, however, as the colonies grow older, thicker filaments begin to appear in the cultures, these are often somewhat twisted, and the threads divide spontaneously after some time's

Fig. 22.
Actinomyces Affanassiew Agar. Two days' cultivation at 37° + 6 days at room tp The margin of a colony. Magnificaton × 1000.

growth in just the same way as does the aerial mycelium of the other fungi of the group, that is to say, starting with division of the protoplasm without any primary participation in the process on the part of the membrane. *Transverse dividing lines will be looked for in vain.* See Fig. 22.

The elements arising from this division remain unchanged after their formation, resembling exactly the spores originating in the aerial mycelium of the previously described fungi, except for a somewhat greater difference in the form and size of the individual elements than usually seen in aerial spores. It is a rather difficult task to place this fungus within any of the groups stated in the present work. Unfortunately, it does not appear from *Harbitz & Grondahl's* work, how the substratum mycelium behaved in the fungus described by them which formed an aerial mycelium, so that we are ignorant as to how far the loss of aerial hyphæ coincides with the above described division process. Future investigations may perhaps show that there exists a connection between the two phenomena.

Likewise, it would be interesting to examine whether the statements advanced by, for instance, *Waksman*, concerning the fin-

ding of sporeless fungi belonging to this group, are correct, or whether the fungi would in effect be able to form aerial mycelium when cultivated on a suitable medium, for instance water agar.

In case these fungi should prove unable to form aerial mycelium, it would be interesting to see whether they present a similar spontaneous division of certain filaments of the substratum mycelium.

GROUP II.

This group comprises fungi which differ from one another to a greater extent than did the fungi of Group I, but on the other hand they have so many features in common that it will doubtless bè most practical to unite them under one head. The group may then be divided into two subgroups, according to the presence or absence of aerial hyphæ. In certain of the fungi these aerial hyphæ are however so slightly developed that it may in a given case be difficult to decide whether a fungus should be reckoned to one or other subgroup, and we had better not attach too much importance to these aerial hyphæ from a systematic point of view.

On the one hand, we find fungi which on several points resemble those of Group I, while, on the other hand, some present no similarity at all, or only a very slight one.

We shall then divide this group of fungi into two subgroups, a and b.

GROUP II A.

One of the grosser features common to the fungi of Group II a, is the formation of an initially undivided substratum mycelium, which, at an early stage, constantly forms aerial hyphæ. Later, the substratum mycelium as well as the aerial mycelium divide by means of transverse septa into unequally sized segments, which, when transferred to a fresh medium, may germinate into a new mycelium.

A characteristic feature in all these fungi is a more or less pronounced polymorphism. While the mycelium of Group I fungi is nearly always regular, it is here very often extremely irregular of form, the colonies frequently presenting a most heterogenous picture.

The main feature by which the fungi of Group II are distinct

from those of Group I, is the spontaneous segmentation of the substratum mycelium. This segmentation is very differently pronounced in the various representatives, in consequence of which the demonstration of it becomes more or less difficult.

The fungi in which this segmentation is least pronounced resemble Group I fungi to a greater extent than those in which it is very conspicuous. The various representatives may therefore be arranged in a consecutive series showing a fairly even transition morphologically, so that, at the one end we have fungi which partly resemble Group I, at the other end, such as have nothing at all in common with that group. As already previously stated, these relatively gradual transitions between the forms cannot form the basis of conclusive inferences as regards a closer or more distant genealogical relationship between the fungi. Not until it has been demonstrated that transformations from one morphological type to another do take place, will the difference referred to above come to play a less important rôle for classification.

In the following section we shall first describe those fungi which exhibit the greatest resemblance to those of Group I.

GROUP II A.

Actinomyces corneae III. Löwenstein (Kràl).

The cultures of this fungus somewhat resemble those we knov from Group I, on account of the formation of a usually very profuse aerial mycelium, in contradistinction to the other fungi of this group. Microscopically, however, they display several divergencies from Group I.

In subcultures sown from an older culture it will be found — on observing the surface of the medium after some time's incubation — that the disseminated small spherical or rod-shaped elements will have increased considerably in size, far more than was the case with Group I fungi. The initial cell often grows to a very considerable size, and frequently this addition to its growth is continued even for some time after the sprouting of the first mycelial threads has begun. These threads are usually somewhat irregular of shape, but, compared with the other representatives of this group, the mycelium must be said to be but slightly polymorphous. See Fig. 23. In the germination it is mainly the more uniform round forms or short rods that form a mycelium, while the great majority of shorter or longer threads and rods do not form the basis of new colonies.

As soon as a small mycelium has formed, frequently while the threads are yet quite short, the first aerial hyphæ arise; this is the case *on rich as well as on poor nutritive media.* This early formation of aerial hyphæ is characteristic of the whole subgroup, it being a matter of indifference which medium be applied for cultivation; in this feature Group II fungi sharply distinguish themselves from Group I, since the fungi of the latter group quite constantly do not form their aerial hyphæ until at a time when the

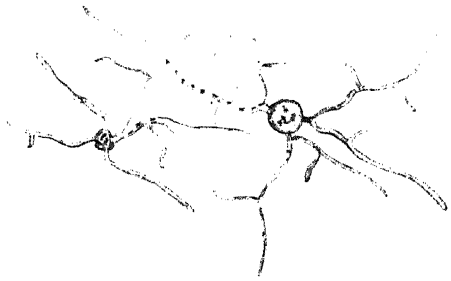


Fig. 23.
Act. corneae. Löwenstein. On 3% glycerine agar for 3 days at room tp. Immersion lenses. Magnification × 1000.

substratum mycelium has reached a considerable size, usually not until after several days' growth.

Besides by their early incidence, the aerial hyphæ differ from those of Group I by not being morphologically distinguishable from the filaments of the substratum mycelium when examined with immersion lenses. While the aerial mycelium of Group I fungi was constantly of a higher refractive power, and the individual threads thicker, than those of the substratum mycelium, *the fungi of Group II present no discernable difference between the filaments of the substratum and aerial mycelia.*

Furthermore, the aerial branches in Actinomyces corneae arise in a manner different from that of Group I fungi. The first aerial threads always arise centrally, and, according as the substratum mycelium increases in size, a steadily increasing number of aerial hyphæ arise more and more distally, decreasing in length towards the periphery where they are youngest and therefore quite short. *We have therefore an even distribution of aerial hyphæ all over the colony,* whereas the aerial mycelium in Group I fungi was

usually seen to arise either centrally, or in the periphery, here often in ring formation, or in both places simultaneously and equally developed.

This fungus produces, as stated, a rather strongly developed aerial mycelium, and yet the filaments are usually far shorter and more rigid than in Group I fungi. This difference is however not nearly so pronounced in this fungus as in the following ones.

After the lapse of some time, towards the stage when the devel-

Plate 24.
Actin. corneae. Agar. 1 month at room tp. Immersion lenses. Magnification × 1000.

opment of the colonies is about to stagnate, a spontaneous division occurs in some of the filaments both in the substratum and aerial mycelia, quite different from the behaviour of Group I fungi.

This division begins centrally on the mycelial filaments, by the formation of septa, growing inwardly from the periphery of the mycelial filaments. It takes some time's practice to detect these extremely fine transverse division lines in this fungus, on account of the minuteness of the mycelium.

This division being accomplished, a displacement will generally occur within the substratum mycelium of the segments that have been formed, so that these become situated at different angles to one another. See Fig. 24. The spore formation observed in Group I is thus not seen here at all; the segments are usually very varying in size and shape, without there being any definite element among them that differs from the others in shape or refractive power. The shortest forms are usually the more homogenous ones, and they most frequently give rise to new colonies in subculture on fresh media. In numerous of the threads, both of the substratum and aerial mycelia, segmentation is absent, or only

slightly pronounced. In Actinomyces corneae we find the same granulation of the protoplasm as we know from Group I.

By *cultivation in broth*, the fluid remains clear, a minute flaky growth appearing primarily at the bottom, *and, very early, small surface colonies which, likewise early, form aerial mycelia.*

This early surface growth is characteristic of the entire group, and affords a good recognition mark from Group I fungi.

The surface colonies in broth are morphologically identical with the colonies on solid media. In the small flakes at the bottom of the tubes the filaments are usually markedly polymorphous, dividing into segments of very irregular shape. (Figure 25).

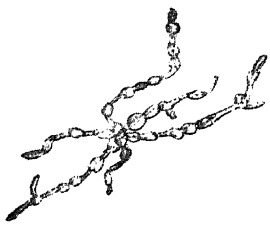


Fig. 25
Act. corneae. Löwenstein. Cultivated in broth at room tp. Magnification × 1000.

Actinomyces Sabrazès & Rivière. (Institute of General Pathology. Copenhagen).

This fungus is somewhat more polymorphous than the preceding one. The colonies adhere strongly to the medium but differ in appearance to some extent from the colonies of Group I fungi: while, in the latter group, the colonies were seen, for instance on broth-peptone-agar, to slope evenly to all sides, and, under low magnification, appeared to be surrounded by a broad transparent zone consisting of long branching filaments, the colonies of Act. S. & R. early become arched in their central portion, the peripheral slightly transparent area of the colony being quite narrow. Frequently the colonies assume a very uneven »humpy« appearance. The colour is somewhat varying, even on the same medium, most frequently yellowish of a reddish hue. *Macroscopically, the white »powder« we know from Group I, is never observed.* The aerial hyphæ are here manifested only by a dry appearance of the colonies. On growing older the colonies become less hard of consistence and are easily crumbled by pressure.

Just as in Act. corneae, the initial cell shows considerable swelling prior to and simultaneous with germination, and, likewise, the first aerial hyphæ appear at an early stage on all media.

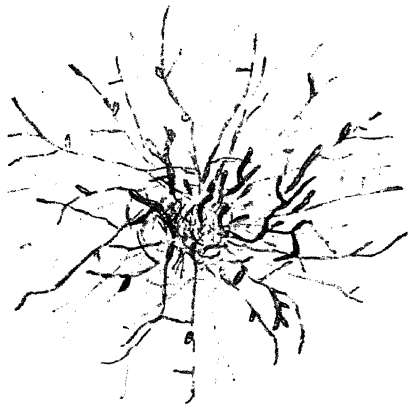


Fig. 26.
Act. Sabrazès & Rivière. Cultivated on agar at room tp. 2 days. Immersion lenses. Magnification × 700.

Occasionally, a primary unicellular mycelium is formed, similar to what we are accustomed to see, but, in certain cases, under apparently identical conditions, the newly formed mycelium di-

Fig. 27.
Act. Sabrazès & Rivière. Agar. Room. tp. 3 days. High power dry lenses. Magnification × 1000,

vides early into large oval or round cells, which sprout into thinner regular threads. Occasionally this early mycelial division is continued a long distance along one individual thread. The swollen cells and the thicker threads are frequently the site of a granulation, differing from that of Group I in that it does not origi-

nate from a disintegration on the part of the protoplasm into unequally sized fragments that again divide into smaller granules. Here, the small granules appear scattered in the highly refractive filaments. Act. corneae also exhibits similar granules, if however less pronounced, see Fig. 23, whereas we find them still more distinct in certain of the subsequent fungi, especially pronounced in such of them as form colonies of lively colours. The colour of the colony is possibly associated with the formation of these granules. On staining a fungus which presents these granules, for instance, by Gram's method, we do not see these granules, such as was the case with the previously mentioned protoplasmic granules, as these granular filaments usually absorbed the dye diffusely. Fig. 26 & 27.

The aerial mycelium has quite the same mode of formation in Actinomyces S. & R., as in Act. corneae. In Act. S. & R. the aerial hyphæ never grow to any considerable length and are usually very irregular of shape. In older colonies, remarkably few aerial hyphæ are sometimes found, which is due to the circumstance that some of the filaments have fallen down on the medium, and others may have become intertangled, so as to form thick stems protruding from the cupolar surface of the colony as thick spikes. Examination of the central portions of the colonies is rendered difficult at an early stage, just as in the case of several other acid-fast ray fungi*) by the formation of a fatty substance outside the threads, which makes it difficult to distinguish them from each other.

Similarly to Actinomyces corneae, both the substratum and aerial mycelia divide into irregularly sized segments by septation.

Surface growth appears at an early stage in broth, the development being on the whole like that of Act. corneae. Fig. 28 shows a

*) No more than in the previous sections do I here enter further into the various tinctorial or physiological characters of the fungi except where these relations bear directly upon the morphology. It is of course of the greatest interest to gain a closer knowledge of these various relations in the fungi, but I found that the purely morphological relations called for primary elucidation. Later, attemps can be made at a subdivision of the groups set up here, in accordance with specific physiological characters. Probably, for this purpose, we shall have to follow other courses than those customarily applied in bacteriology. At any rate, the ordinary methods have, so far, yielded but a poor help for the purpose of classification of these fungi, nor have the immunobiological reactions proved of any avail in this respect.

picture of the fungus such as it appears at the bottom of broth. Cultivation on $^1/_2$ % caffein agar, and 2 % lithium chloride agar gives but poor growth, the mycelial filaments remaining quite short. Morphologically the picture shows nothing of special interest.

Fig. 28.
Act. S. & R. Cultivated in broth at room tp. 14 days. Immersion lenses. × 1000.

Streptothrix Deycke (Kràl).

The cultures present an appearance almost identical to that of preceding fungus. The colour is usually an ochreous yellow with a reddish hue. The colonies adhere rather strongly to the medium

Fig. 29.
Streptothrix Deycke. Cultivated on agar at room tp. 1 month Immersion lenses. Magnification × 1000.

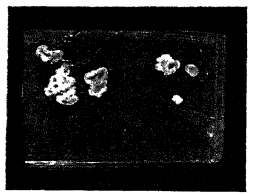


Fig. 30.
Act. Eppinger. Cultivated on agar at room tp. 14 days.

but are however fairly easily subdivided. There is a sparse growth of aerial hyphæ, not visible to the naked eye. All that has been said of the morphology in Actinomyces Sabrazès & Rivières applies also here, except that these fungi are somewhat more polymorphous and show a more abundant segmentation, which renders the demonstration of the septa easier. Fig. 29 represents the marginal portion of an older colony on agar. The irregularity of the individual segments is conspicuous.

In broth surface growth begins early, initiating as small isolated scales, which, when growing in size, readily sediment in the tube by shaking. The morphology is here, too, like that of preceding fungus.

The three following fungi resemble one another very much and behave morphologically as Streptothrix Deycke.

Actinomyces Eppinger (Kràl) forms humpy colonies (see Fig.

Fig. 31.
Actin. Eppinger. Cultivated on ágar at 37° for 24 hours. Immersion lenses. Magnification × 1000.

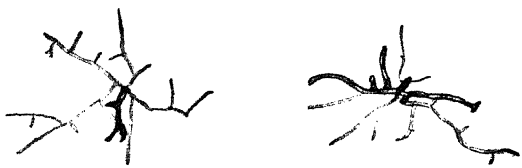


Fig. 32.
Cladothrix asteroides Eppinger. Broth peptone agar culture. 16 hours at 37°. High pover dry lenses. Magnification × 700.

Fig. 33.
Same as preceding observed with immersion lenses. Magnification × 1000.

30) on solid media. Most frequently the colonies adhere but loosely to the medium. This difference in adherence is primarily due to the varying extent to which the fungi send mycelial filaments down into the medium, and, as far as the fungi of this group are concerned, this may vary a great deal even for the individual fungus, in

contradistinction to what was seen in the case of Group I, where the colonies are constantly strongly adherent. The colour of the colony is almost the same in this and the subsequent fungi, very pale rose with a yellowish hue.

Aerial hyphæ are formed at a very early stage, while segmentation of both aerial and substratum mycelia, which, as usual for this group, initiates centrally — has a late incidence and is less abundant than for instance in Cladothrix Eppinger.

Fig. 31 shows a young mycelium with similar granules in the protoplasm as those encountered in Actinomyces Sabrazès & Rivières, and with the same early occurring septa in the mycelium. One of the threads seen in the picture, originating from the large initial cell, is an aerial branch without it being possible by this method of examination to distinguish between this and the other mycelial threads.

Nocardia Eppinger. (Institut Pasteur).

Very similar to preceding fungus, except for more pronounced polymorphism and a somewhat slower rate of growth.

Cladothrix Eppinger. (Král).

Resembles the others, but has a considerably more rapid rate of growth than the latter two fungi. Usually the colonies do not adhere to the medium and, if there has been a dense inoculation, the individual colonies will be displaced towards one another so as to form irregularly arranged foldings and ridges.

It is somewhat easier to study the morphology of this fungus than that of the preceding ones owing to its more rapid growth, and, to the fact that nearly all the mycelial filaments divide into segments almost all along their course.

Germination occurs in the usual manner. Figures 32 and 33 represent young mycelia observed under high-power dry lenses and immersion lenses, respectively. On the first picture the early formed aerial hyphæ are seen.

These segments very readily break from one another, and it is clear that smear preparations of such a culture will easily produce the impression upon the observer of being a colony of rod-shaped bacteria. Fig. 34, finally, shows some mycelial filaments at the stage of septa formation. We shall here mention some features of the morphology because they are especially easy to follow in this

fungus. The same features are moreover to be found in all the other fungi of this group, but usually much more difficult to study. We see here the long slender threads, which are frequently fairly regular in this fungus. Besides, we see along the course of the

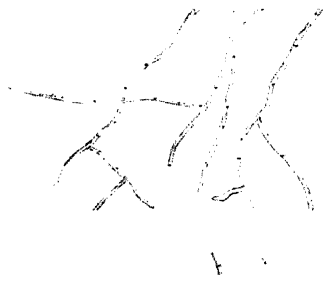


Fig. 34.
Clad. asteroid. Eppinger. Cultivated on 2% water agar at room tp. 2 days Immersion lenses, Magnification×1000.

threads a series of darker granules and some transverse lines. The granules are the initial stage in septa formation, being always situated in the wall of the threads, the septum dividing the mycelium arising here. When the septum has been formed, a displacement will frequently occur. as shown in Fig. 35, the continuity of the threads being not totally disrupted, as the segments remain

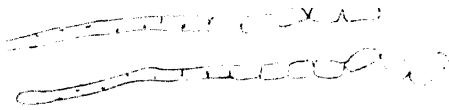


Fig 35.
Schematic drawing of the transverse divsion of the threads in Cladothrix asteroid. Eppinger. Magnification × 2000.

attached to one another in a very small area along the course of one thread wall. As soon as the segments become detached from each other, they show perfectly rounded outlines, a fact, which presumably indicates the occurrence of an increased internal pressure within the individual cells, which perhaps has the secondary effect of displacing the individual elements in their relative situations.

How far this frequently observed attachment between the segments along the one wall should be associated with the above mentioned mode of formation of the septa, it is of course difficult to

ascertain; it would however seem reasonable to imagine a connection between these two phenomena, so that the incomplete detachment should be ascribed to the circumstance that the splitting of the septum, which must take place prior to the relative displacement of the elements, is not always so complete as to totally part the threads into transverse segments. Such as it is the case, for instance, with most of the rod-shaped bacteria, where the dividing septa apparently arise along the entire periphery of the rod, whence they spread towards the centre. We shall in the following get ample opportunity of finding similar relations in some of the subsequent fungi.

In broth this fungus grows like the previously mentioned fungi, showing constantly an early, abundant surface growth.

Streptothrix rubra (Krâl).

This fungus might with equal right be classed with Group II b, on acconnt of its inconstant and sparse formation of aerial hyphæ.

Otherwise there is perfect correspondence, morphologically, between this fungus and Streptothrix polychromogenes.

Strept. rubra has a more rapid rate of growth than the previously described fungi, its development being usually accomplished in the course of a few days at 37°, at any rate, in the main features.

The colonies always adhere quite loosely to the medium so that they may be removed as readily as for instance a colony of diphtheria bacilli. In young cultures the colonies have a dry appearance, later they assume a more moist sheen. The colour of the colonies is often a beautiful cinnabar red. After long desiccation on filter-paper, the first colonies arising from dissemination of these desiccated germs, may be somewhat decolorized; the red colour will however always be recovered in a second transfer.

When cultivated in broth, surface growth always appears at an early stage, soon covering the entire area of the broth and even extending upwards along the glass of the tube. The rate of this surface growth may be somewhat protracted by employing for inoculum germs that have been exposed to desiccation for some months. In this case small firm spherical colonies may arise primarily at the bottom of the tube, surface growth not appearing until after 4 to 5 days.

In the previously described fungi we have constantly observed

that they were usually very polymorphous, without however entering further into the question of the nature of this polymorphism.

Streptothrix rubra, being one of the most polymorphous of all the ray fungi, is well adapted for a closer study of the irregular forms that may arise in these fungi, the reason why I have postponed mention of these up to this point.

The course of development of this fungus on solid medium, for

Fig. 36.
Streptothrix rubra. Cultivated on agar at 37° for 24 hours. Immersion lenses. × 1000.

instance ordinary broth agar, when inoculated from a few days' old culture, is the following: the disseminated elements, which are nearly all of them spherical, oval, or short cylindrical, of a homogenous appearance, will increase somewhat in size prior to germination such as it was also seen in the preceding fungi, and first after 5 to 6 hours' incubation at 37°, when they have grown to about double their initial size, do they give out one or several strongly refractive sprouts. At ordinary room temperature germination does not take place until after the lapse of far longer time.

Under the process of germination and continuing during the development of the mycelium within the first twenty-four hours, the initial cell will usually grow in size, while it preserves its round or oval shape; in this way the initial cell may grow to a size somewhat exceeding that of a red blood-corpuscle.

When the newly-formed mycelial threads have grown to some length, they begin to develop large swellings, usually simultaneous with the occurrence of septa in the mycelium, as a rule centrally on the mentioned swollen areas. These swollen areas, which possess the same refractive power as the other areas of the mycelium when viewed under immersion lenses, may be extremely varying in form; most commonly they are spherical, club-shaped or spindle shaped. Fig. 36 shows such a young mycelium.

From these swollen areas one or several regular, slender or slightly bulbous threads now begin to sprout, and these may again, after the lapse of some time, become the seat of similar swellings which are again able to form new threads. Occasionally

Fig. 37.
Streptothrix rubra. Cultivated on agar at 37° for 36 hours. × 1000.

some of the swollen areas divide primarily by means of a whole series of septa that have been formed in them, so as to produce an image resembling the one we know from the diphtheria bacillus as »banded« forms. See Fig. 37. At this stage certain of the threads

Fig. 38.
Streptothrix rubra. Cultivated on agar at room tp. for 4 days. Immersion lenses. Magnification × 1000.

divide into unequally sized segments, while other threads sprout from the large swellings, these not dividing into segments in the usual manner until they have reached their final length. This synchronous incidence of large swollen forms, long slender threads, and rows of shorter or longer segments, produces a very heterogenous picture. See Fig. 38. When growth is about to stagnate,

the picture becomes less polymorphous. Nearly all the threads divide into segments, which again divide into smaller and smaller segments, frequently displaced in their position to each other in a similar way as we have previously seen, and finally we get a fairly monomorphous picture. The colonies now consist almost exclusively of small short rods or coccus-like elements, only some few isolated swollen forms being left. (See Fig. 39).

Fig. 39.
Streptothrix rubra. Cultivated on 1/2 % glucose agar for 4 days at room tp. Immersion lenses, Magnification × 1000.

The less rich in nutritive substances the medium is, the smaller do of course the colonies become, and the less polymorphous. This is however more a quantitative than a qualitative difference, as we shall see below.

The small elements that have arisen by the described segmentation, may now finally, in certain cases, exhibit an independent growth in the same culture in which they have arisen. This growth is however restricted to the sprouting of an isolated short thread, which divides under relative displacement of the individual segments. When the colonies have reached this stage, one should take the utmost precautions on examining the preparation under cover glass with immersion lenses, not to disturb the natural arrangement of the elements within the colonies. For, if care is not taken, one may obtain an impression of colonies without any special arrangement, showing the strongest resemblance to staphylococci. It is therefore evident that it is extremely difficult to form an impression of the morphology of such a fungus from the usual smear or suspension preparations.

The morphology here described has been traced step by step in all the fungi examined, the only difference between Streptothrix rubra and the preceding fungi being its especially beautifully pronounced polymorphism.

The small final cells are thus seen to arise from the large swollen forms, the latter forming a transition link. These swollen forms, which have of course also been observed by previous investigators and usually designated as clubs, have been the source of much discussion. The designation »involution forms« has stuck to them, because most authors have taken the regular threads to be the »normal« elements.

It might be appropriate in this connection to discuss the term »involution« a little further.

For this purpose we shall have to consider the origin of the term and try to make clear what it signifies. The latter task is however extremely difficult, because it proves that this term, in every-day use by bacteriologists, is applied in many different senses, which just explains its general applicability.

The tenacity of life shown by this term is hard to understand, considering the conditions of its origin. For it dates as far back as the younger era of bacteriology, when the constancy of bacterial species was as yet a question of controversy.

After violent contests it was finally vigorously established that the bacterial species and their morphology were something very nearly absolutely constant, the bacteria being, according to their morphology, divided, on rather narrow lines, into cocci, bacilli and spirilla. Cocci were cocci, and bacilli were bacilli, and all variations were considered, so to speak, as being of evil. The variation forms which, nevertheless, did appear now and then within the practice of every bacteriologist, had the term »involution forms« attached to them, the cause of their coming into existence being taken to be external deleterious influences. These diverging forms were encountered especially in old cultures, and, since, besides being morphologically divergent, they furthermore proved to be poorly staining, it was inferred, and doubtless frequently with perfect right too, that the forms in question were degenerated and unable to multiply. The latter relation, the power of reproduction, has been but slightly subjected to investigation, although it would seem to be of vital interest.

But, besides these divergent forms in old cultures, numerous bacteria and, we dare say in a more pronounced degree, certain of the ray fungi, frequently display forms whose only sign of degeneration is their »divergent« morphology.

These forms are however likewise termed »involution forms«,

even though the opinions of most of the authors who have occupied themselves with these questions, seem to concur in that numerous of these forms arise under conditions that would not seem especially adapted for producing abnormal forms.

And, moreover, bacteriologists who have performed the only rational experiment, that is to study whether the atypical forms are viable and how they develop, arrived at the result that the atypical forms were viable.

Emil Chr. Hansen may be mentioned among these investigators as being one of the first to subject the peculiar forms sometimes displayed by Bacterium aceti in vinegar to a closer study, with the result that the atypical forms in no other respect than that of morphology showed anything abnormal.

Another investigator, who has decidedly carried out some of the most beautiful researches on bacterial morphology, is *Ernst Almquist.* Most laboriously he followed the origin and development of the peculiar forms encountered in certain typhoid, dysentery and cholera strains, finding these to be perfectly normal as regards power of reproduction.

Besides these, *Metchnikoff* should be mentioned as the first to demonstrate the branching bulbous forms in Bacillus tuberculosis. He was succeeded by a whole series of investigators who found similar forms, partly among the acid-fast bacilli, partly in diphtheria bacilli and several others.

Finally, *Hankin & Leumann's* work on Bacillus pestis, showing its characteristic morphology when cultivated on agar containing a certain per cent of NaCl, gave the impetus to several other works among which may be named *Matzuschitas* and *Maassen's* on the morphogenetic influence of a number of different salts on many various bacteria.

The conclusions arrived at by the various bacteriologists, are very diverging; while some consider the atypical forms as adequate variations, others are more bold in their inferences, as for instance *Metchnikoff*, who interprets the branching forms as a sort of atavistic forms indicating that the tubercle bacillus should phylogenetically belong to the higher differentiated branching fungi, and *Almquist*, too, thinks it reasonable to conclude from his findings that certain bacteria have a cycle of development resembling that of higher differentiated fungi.

But, notwithstanding these works and numerous others pointing

in the same direction, the forms found by these authors, belonging to widely differing bacterial groups, are constantly included under the same collective term, involution forms.

This term thus implies all morphological divergencies from the form once and for all established as the »normal« one.

A typhoid bacillus is a rod-shaped microbe, and, if, for instance, it appears as a thread, well, then it is due to inhibition of its normal transverse division, and, if it appears as a spherical body, it is due to inhibition of its normal longitudinal growth.

And, likewise, if diphtheria bacilli form clubs and branching forms, the occurrence of these is ascribed to unfavourable external conditions; and again, if bulbous forms arise in the ray fungi — in which branching forms are allowed — such are also said to be due to inhibition of the normal longitudinal growth.

This mode of consideration: let us not have any disturbance of the good order of things within bacterial classification — was fully understandable when bacteriology was still in its infancy, at a time when the vehement contest as to the constancy of the morphological and physiological characters found in the bacteria, had just been successfully settled to the effect that bacteria do not at any given moment change in one or other direction; and, the view becomes the more understandable yet, when we bear in mind that there existed at that time only a very scant empirical material in this field of work.

But, at the present moment, when nobody is thinking of disturbing the basic idea that bacteria, derived from one single microorganism, with great tenacity retain the character of their progenitor, and only in extremely rare cases present the so-called »mutation« forms, and where, moreover, with each new day, as it were, it is discovered that bacterial species, the individual strains of which were formerly considered as identical, can be differentiated into »types«, each of which tenaciously keeps its specific character, one would think that this »lumber-room« term, »involution-forms« could be taken up for serious investigation, so that it might be made clear that it is widely differing things that go under the same designation, and that, as far as many of the forms are concerned, it is utterly misleading.

»Atypical« forms are of frequent occurrence among the members of the groups of Ray Fungi.

It has been taken for granted, once and for all, that the bran-

ches of these fungi should be of uniform thickness, and, if spherical or bulbous elements were formed, these were ascribed to deleterious environmental conditions manifest by inhibition of the longitudinal growth, the result of which being »involution« forms.

Lieske, who is the author who most recently and most elaborately has treated the question of these forms, arrives at the same result. He defines involution forms as forms differing from the normal growth-forms, writing as follows: »in allen Strahlenpilzreinkulturen findet man häufig die Ende der Fäden kolbig verdickt..... Sie stellen lediglich eine Verbreiterung des Strahlenpilzfadens infolge durch äusseren Einflüsse verminderten Längenwachstums dar. An geigneten Kulturen sind die kolbige Verdichtungen grampositiv wie der lebende Faden, in frische Nährlösung gebracht wachsen sie in normaler Weise weiter.«

Having told us that the bulbous forms are due to inhibition of longitudinal growth, in consequence of which they are to be found at the tips of the threads, he goes on to state that they may also occur along the course of the threads, so that, if we are to retain the hypothesis of inhibition of the longitudinal growth, this inhibition must therefore have been overcome at one or other stage of the development. In several places *Lieske* emphasized that these forms are found especially in old cultures, and yet, most of his illustrations just show them to arise in young cultures.

As for the mode of formation of the swollen forms, *Lieske* writes furthermore: »Die Fäden schwellen zunächst an einem Ende kolbig an, die Anschwellung vergrössert sich nun mehr, so dass schlieslich eine grosse Kugel entsteht an der eine Teil des ursprunglichen Strahlenpilzfadens noch unveränderlich stielartig aufsitzt. Interessant ist die Weiterentwickelung solcher Formen auf normaler Nährböden. An einer oder mehreren Stellen der Kugeln entstehen dann Ausstülpungen die zu normalem Fäden von gewöhnlicher Dicke auswachsen. — Bei Actinomyces polychromogenes wurde beobachtet, dass die nach 24 Stunden bei 37° auf fünfprozentigen Ammonchloridagar entstandenen kugeligen Involutionsformen nach weiteren 48 Stunden auf dem selben Nährboden in der erwähnten Weise weiterwuchsen was ohne Zweifel daraus zu erklären ist, dass das Plasma sich in dieser Zeit an die *Giftwirkung* des Ammonchlorids gewöhnt hatte so dass dies das Längswachstum nicht mehr hemmen konnte.«

As the here mentioned Streptothrix polychromogenes, which is also the one from which the main portion of »involution« forms presented in *Lieskes* illustrations was derived, *has* also been examined by me (see later) and as it proves to be morphologically perfectly identical with Streptothrix rubra, it is possible to compare *Lieske's* and my own results; it also proves that the pictures resemble one another, only *Lieske* has not quite realised the internal structure of the colonies; and I shall willingly admit that there are almost insurmountable difficulties in the way, when employing the usual methods of examination.

One thing that surprises me in *Lieske's* results, is that the swollen forms should be especially abundantly represented in old cultures. This agrees so badly with my own experiences with those of the fungi examined which, at all, exhibited such forms — (as was seen they were of very rare occurrence in Group I) — that I feel apt to believe *Lieske* has been prejudiced in his conception by the current view as to the etiology of these forms.

For, as it appears from above investigations, these forms are chiefly found in cultures that are in full development, themselves participating lively in this development.

As we have likewise seen, these forms occur on ordinary broth-peptone-agar of neutral reaction (litmus-paper) at all temperatures at which the fungus grows at all. And, quite similarly, they occur on a 2 % water agar and broth agar to which is added glucose in varying quantities, or magnesium sulphate, lithium-chloride or caffein. We may also find them on glycerine agar and ascitic agar, in short, in the most different media, only in somewhat varying numbers. The course of development is however identical on all the media.

In a 2 % water agar, on which the colonies remain quite small, we find, for instance, the same swelling of the initial cells, whereas the swollen areas of the mycelial threads are but little pronounced, because the short threads divide early into small final cells, at which stage the development is terminated in this poor medium.

As stated, *Lieske* was of opinion that is is especially in *old* cultures that the atypical forms arise, an assumption that is moreover accepted from numerous sides. We must here make clear what is meant by *old*. When is a culture old?

Well, a culture of coli bacilli which has grown at 37° for instance for 4 days, is extremely old, while a culture of tubercle

bacilli of the same age is very young, if it has at all begun to grow.

Generally speaking, a culture may be said to be old when its development has stagnated, or is about to do so. It is of great importance, — and not least when dealing with ray fungi, — to realize that this phase of stagnation has its incidence at very varying dates, so that it is not feasible, without further comment, to compare a culture grown at room temperature with one cultivated at 37°. While thus development may have ceased at 37° in the course of 3—4 days, more than double that time may elapse before it will have ceased at room temperature.

A more or less liberal dissemination of the germs also plays a part here; if the culture be densely sown, the development of the individual colonies will cease at a much earlier stage than when the colonies lie scattered; and here, where we have a cycle of development, the image that appears will become quite different in the course of the same period. A dense culture becomes old at an earlier date than a scattered one. The same difference appears according to the varying nutritive value of the medium. In a medium rich in nutritive substances development continues for a longer period than in a poor one, all other conditions being equal.

It will be readily understood that if one does not constantly bear these facts in one's mind, the results may become astonishing. For instance, on examining a culture of Streptothrix rubra after 3 days' growth on agar at 37°, we find exclusively short forms as described above, while the same fungus, on the same medium, but cultivated ar room temperature, displays the most excessive polymorphism. If we accept the old hypothesis of involution forms, as being due to inhibition of growth, we get at the peculiar result that agar should be extremely »poisonous« at 18° and harmless at 37°. Examining however the room culture after a week, the picture proves to be identical with the one we had in the course of 3 days after incubation at 37°. The inhibitory effect has now then been overcome also at room temperature! And considering the development within the individual colony, where the long regular filaments were seen to arise first from the large swollen initial cells, and later from the swollen areas in the threads, this would have to be explained, according to the current view, as the result of a struggle between the fungus, fighting to gain its normal shape, against the noxious effects of the medium, the which it also conquers at last, after repeated fights.

Nay, the explanation is no doubt that these fungi are constitutionally extremely polymorphous, this being just the most characteristic feature of their morphology.

On the whole, it applies to the forms described here, as well as to the very peculiar forms we may encounter in the bacteria, that the only *reliable mark to indicate that a form is abnormal, is, that it has lost its power of development.* Therefore, I hold that one should always postpone one's judgment as to the nature of the atypical forms, until investigation has been undertaken in regard to this question. I feel convinced that several bacterial forms which now go under the designation »involution« forms, will be seen in quite a new light after such an investigation.

As for the viability of the forms here dealt with, we saw that they participate lively in the development of the organism and also continue to grow when transplanted to a fresh medium, that is to say, provided they are young forms. The older forms, which often appear vacuolated and slightly refractive, will, in transfers, break up into small granules that fail in power of germination. This, of course, justifies our concluding that these forms are degenerated, but, as they have already contributed their part in the development of the ray fungus, this degeneration is quite natural and does not speak for the supposition that these forms should be less normal than, for instance, the regular threads; these, too, as has been frequently referred to, undergo a protoplasmic disintegration with age, which reduces their viability.

As for the question of the appearance of the fungi on different salt-containing media, we may say, that the morphological picture appearing on such media quite resembles those we know from media without addition of these salts, only the development often ceases at a very early stage, because the conditions are unfavourable, so that the large forms we know from the young cultures may predominate. In media on which the development is continued, there occurs no divergency in the course of development, only the polymorphism is frequently still more pronounced than in media without addition of salt, and, at the same time, the forms may appear slightly exaggerated. In a $^1/_2$ % caffein agar, the forms become early vacuolated, the development being limited to the swelling of the initial cells and the formation of short swollen germ threads. Already after 48 hours' growth at 37°, these organisms fail to grow, on the germs being transplanted to a fresh medium.

We shall delay comparison between the fungi described in this section and those described in the literature, which belong here, until we have described the fungi in the following section, and likewise we postpone a further valuation of the spore nature of the small final cells of these fungi until then.

GROUP II B.

The representatives of this group, in distinction from those of Group II a, form no aerial mycelium.

Otherwise there is no sharp boundary between this group and the preceding one. A fungus like Streptothrix polychromogenes resembles very much Streptothrix rubra, the sparse growth of aerial hyphæ that may be found in the latter being no doubt of but slight value for classification, so that Streptothrix rubra might in effect, with equal right, be classed with the group now dealt with. But, as long as it has not been demonstrated in which way transformations take place — if such do at all occur — we had better undertake the classification on somewhat stricter lines.

Streptothrix polychromogenes Vallée (Kràl) is a saprophyte, first found and described by Vallée (1903).

Its morphology corresponds fairly well with *Vallé's* description.

The colour of the colonies is a beautiful red; occasionally, however, the red colour may, in individual colonies, be replaced by an ochreous yellow, which may be preserved through several generations (morphologically the red and the yellow colonies are perfectly identical). The colonies constantly can be easily removed from the medium and are of a soft, pasty consistence.

In broth there is early surface growth, rapidly covering the entire surface area. The least shaking of the tubes will readily cause small scales from the pellicle to sediment in the broth, the fluid remaining clear.

After long time's desiccation on filter paper, a primary bottom growth in the form of small firm spherical bodies may arise, just as it was seen in the case of Streptothrix rubra; in the course of

a few days, however, the first scales will appear on the surface as well. As has been said, this fungus presents the strongest points of resemblance to Streptothrix rubra.

In subcultures on fresh medium the initial cells swell considerably previous to and simultaneous with germination, this fungus showing as pronounced polymorphism as Streptothrix rubra (Fig. 41).

Examining, for instance, 24-hour cultures, grown at 37°, the

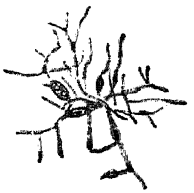


Fig. 40.
Streptothrix polychromogenes. Cultivated on broth agar, 20 hours at 37°. Dry lenses × 700.

Fig. 41.
Streptothrix polychromogenes. Cultivated on broth agar. 20 hours at 32°. Immersion lenses. × 1000.

colonies will usually be surrounded by an aureole of bulbous or club-shaped mycelial filaments. As the colonies grow older, these large forms are usually replaced by long threads, mainly regular in shape, which finally divide into segments, starting centrally; concurrently, the various segments become displaced in their position towards each other, so as to be placed at different angles on one another. The segments are frequently rod-shaped and of unequal length. (Fig. 42). These rod-shaped segments now often elongate somewhat without however forming a new mycelium, showing only slightly pronounced swelling, and finally they divide into still shorter segments, which are frequently almost spherical.

Cultivated on poor nutritive media the growth becomes sparse and the fungus more monomorphous.

For cultures on salt-containing media and on caffein-agar, applies what was said in the case of Streptothrix rubra.

In broth cultures the morphology is quite identical to that ap-

pearing on solid media. Primarily the more polymorphous mycelium is formed, passing into more regular forms, to divide early into small final cells.

Certain mycelial threads in Streptothrix polychromogenes undergo a protoplasmic granulation similar to what was seen in Streptothrix rubra, frequently so marked that the threads assume a peculiar speckled appearance.

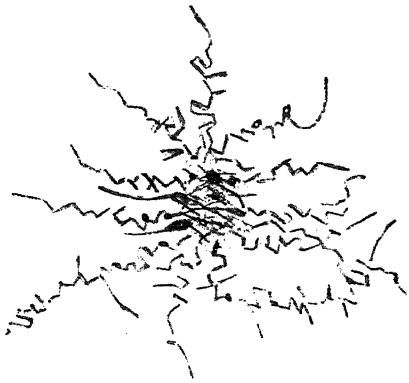


Fig. 42.
Streptothrix polychromogenes, cultivated on broth peptone-agar at 15° for 6 days. Dry lenses. Magnification × 700.

On page 136 we just cursorily refer to *Lieske's* statement that this fungus may form isolated laterally situated spores. I have not succeeded in finding such.

We shall, in this connection, dwell a little on the question as to whether a sexual fructification process may possibly occur in the ray fungi. Earlier investigators never found such, but, in his great work on the Ray Fungi, *Lieske* advances as his opinion that a sexual process actually exists in some cases, and, as the fungus we are dealing with at present is said to display these sexual forms in an especially marked degree, it seems natural to treat this question here.

As mentioned, *Lieske* states that in numerous ray fungi a peculiar spore formation takes place in a rather intricate manner:

»Lange ausgewachsene Mycelfäden biegen sich zuweilen am Ende schwach nach einer Seite um, und an der äusseren Krümmungsfläche entstehen senkrecht zum Mutterfaden in kurzem Abstande zwei Seitenzweige, die nur eine verhältnissmässig geringe Länge

erreichen. Auch der ursprüngliche Faden wächst nur wenig in die Länge. Dagegen wächst das durch die beiden Seitenäste begrenzte Stück des Mutterfadens wesentlich in die Dicke, so dass es ungefähr mindestens das doppelte des ursprünglichen Durchmessers erreicht. Die beiden Enden des Mutterfadens und die beiden Seitenäste stellen sich allmählich in Winkeln von 120 Grad. zu dem verdickten Fadenstück ein. Die von dem verdickten Fadenstück abzweigenden Hyphenenden verlieren nach einiger Zeit ihre Gefärbbarkeit und sterben ab, während das verdickte Fadenstück stark gramfärbbar bleibt. Auf neuen Nährboden gebracht wächst dasselbe zu einem neuen Mycel aus«. (S. 86). *Lieske* does not account for the way in which he followed the formation of these peculiar »Vierhyphensporen«, and, with the technique applied in the present work — which should be especially adapted for detecting such forms — I have never succeeded in demonstrating elements that showed even a superficial resemblance to *Lieske's* spores.

I must therefore join in the opinion of earlier investigators, that no specific sexual fructification process can be demonstrated in the ray fungi.

Nocardia farcinica. (The State Serum Institute, Copenhagen). This is a branched fungus like the preceding one, and likewise polymorphous, if however in a less degree. The appearance of the culture has been elaborately described elsewhere, for instance by *Nocard* himself, so we need not go in for a more detailed description of it here.

It forms a mycelium just in the same way as the other ray fungi described, this mycelium dividing spontaneously into segments in the customary manner (Fig. 43). The segmentation begins centrally, and the mycelium divides into very irregular segments. After the formation of the septa, the individual segments are frequently seen to grow out transversely on the mycelial threads without any displacement on the part of the segments in the mycelial thread. This segmentation of the threads without displacement of the individual segments in their position towards one another, produces another picture of older cultures, than for instance, the one appearing in Streptothrix polychromogenes. The course of development in broth is identical to that on solid media. (See Fig. 44).

This morphology proved to be constant in a series of investigations, and it was quite by chance that I made an experience

in regard to the morphology of Nocardia farcinica which, as will be shown, was in beautiful accordance with several other previously made experiences of the same kind in regard to other branching fungi.

On studying once more *Nocard's* treatise on this fungus at the point of time when I was about to conclude this work, it struck me — what had escaped my notice on the first perusal —, that the description *Nocard* gives of the fungus corresponds but badly with

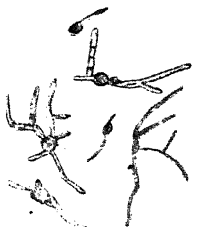


Fig. 43.
Nocardia farcinica. Cultivated on acitik agar. 24 hours at 37°. Immersion lenses × 700.

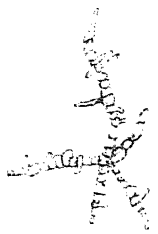


Fig. 44.
Nocardia farcinica. Cultivated on ascitic agar, tp. 14 days. Immersion lenses. × 1000.

the above. He writes about the ramifications: »Cette ramification est plus apparante que réelle; l'etude attentives des colonies récents montre que la developpement des filaments se fait par elongation; lorsque le bacille a aquis le double de sa longueur primitive, il se segmente et l'article nouvellement formé s'infléchit, le plus souvent a angle droit, sur le segment ancien qui continue a croitre en droite ligne; en somme, il s'agit la d'une fausse dichotomisation analogue a ce que l'on observe chez les cladothrix.« As I had earlier noticed this mode of growth, — which *Nocard* erroneously parallelizes with the false branching of the cladothrices — in other branched fungi, I repeated my examinations of Nocardia farcinica, without anticipating any result; but, as good luck would have it, this time Nocardia farcinica actually presented a picture on the broth agar which perfectly corresponded to *Nocard's* description. We shall enter a little further into this interesting phenomenon. (Fig. 45).

This variation in morphology, which strikes one as astonishing, is in fact no unusual finding as far as these fungi are concerned. In »Hospitalstidende«, 1920, I described how a branched fungus be-

came gradually less and less branched, and, in the course of some few subcultivations, almost totally lost its branches, to assume finally an appearance which bore great resemblance morphologically to diphtheria bacilli.

At that time I had not as yet devised the method of technique described in this work, and had therefore to proceed in the usual manner with suspension and smear preparations. Certain information was moreover derived by examining the growth in hanging-drops of broth in moist chamber.

Fig. 45.
Nocardia farcinica. Cultivated on agar, 24 hours at 37°. Immersion lenses.
× 1000.

It might be of interest, now, to look into the morphology of this fungus when examined directly on the medium.

Actinomyces 1920 (Almindelig Hospital). The fungus was isolated in pure culture from sputum derived from a phthisic.

Having obtained the fungus in pure culture, it proved to grow well on all the usual media, the colonies having preserved their appearance ever since. They are whitish or slightly yellowish of colour; round of shape and pasty of consistence. Surface growth occurs early in broth, forming a pellicle from which scales frequently sink to the bottom, the fluid remaining clear.

In subcultures from a few days' old agar culture, the inoculated surface will be seen to be covered by small spherical or short cylindrical elements which, after some time's incubation, begin to germinate, usually by giving out a small sprout laterally on the small primary cell, which almost constantly increases more or less in size during the process of germination. Occasionally several sprouts arise, rarely more than two, however, and usually in diametrically opposite directions. This germination process results in either a club-shaped or fusifor melement, and sometimes even in the formation of a small mycelium. At a very early stage, a discreet dark division line is formed, dividing the thicker portion

of the newly-formed element into two parts; concurrently with the incidence of this septum, the fusiform element will be seen to bend a little, the result being a parting of the two segments, not complete however, since they remain attached to one another at a small area, the presence of which attachment can be inferred, only, by the relative situations of the two elements. For, they are displa-

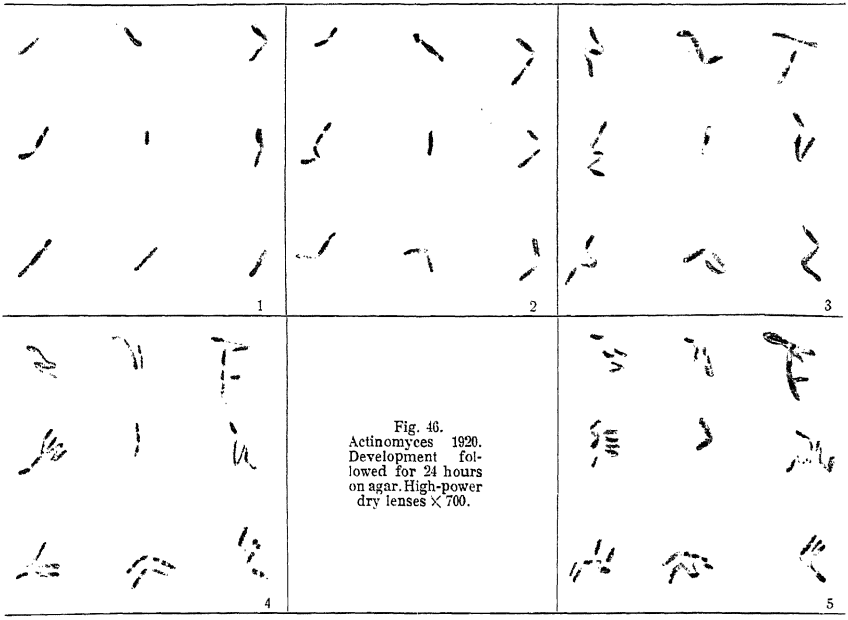


Fig. 46. Actinomyces 1920. Development followed for 24 hours on agar. High-power dry lenses × 700.

ced in their position towards one another in such a way that the two halves form an angle at varying degree. This displacement having taken place, the two »legs« of the angle may continue to grow, and if the »legs« located proximally to one another, elongate, this has the effect that the newly formed cells become able, by mutual pressure on each other, further to displace the »legs« of the angle from which they have arisen. By this means we get pictures shaped like the capitals V, Y or H. As will appear, this mode of growth corresponds perfectly with the one described by *Nocard* for Nocardia farcinica, and also with the one found by myself for the same fungus, and furthermore, it is suggestive of the peculiar displacement of the segments encountered in the filaments of various other ray fungi.

This mode of growth, with which we are going to deal further

below, we shall term »*angular growth*«. It has been previously described in regard to Bacillus diphtheriæ by *Kruse* and others.

This angular mode of growth is a perfectly constant morphological feature in the fungus here dealt with. *As soon as an element is lying isolated, and the new elements resulting from segmentation* are formed, they will constantly be displaced in their position to each other in this way. (See Figure 46).

Is this angular mode of growth a character that may serve as a criterion in classification? Is it quite specific for ray fungi which, like the one here dealt with, have entered into a »bacillary« phase of development.

In order to answer these questions we must primarily make clear whether bacteria, which otherwise show no points of similarity to the ray fungi, may exhibit this peculiar angular growth. *Hill*, already, examined a long series of bacteria with the object of elucidating whether this mode of growth, which he terms »post fission movements« in Bacillus diphtheria, was something specific for this microbe, and he found that this was so. I have myself, examined as many different species of bacteria as possible in this respect; in none of them did I find this peculiar mode of growth except in the fungi mentioned in the following section.

Most rod-shaped bacteria multiply in the following manner: a short rod elongates to become a long rod, or short thread, which divides into two or more segments by means of septa, after which the separated rods are displaced freely in their position to each other; examining a colony of such bacteria, it will be a very rare exception to find two elements situated as if they had developed in the above described manner by »angular« growth.

This mode of growth of course lends a quite specific appearance to the colonies, especially in the case of quite young colonies. Examining under high-power dry lenses, one feels almost sure that the picture represents true branching forms; on tracing the development step by step, it will however appear that the great majority of the »branches« originate from the angular growth.

True branches are however also found in this fungus, the genuineness of which can be readily ascertained by examining under immersion lenses. These ramifications are however usually very few in number, and only very rarely do we meet a picture like that of Fig. 47, showing the growth of young colonies of Actinomyces 1920 on Glycerine agar, the inoculated germs being derived

from an extremely old culture. We may occasionally encounter similar colonies on other media too, but in subcultures the fungus will rapidly lose its power to grow in long branching threads.

In young cultures the elements are generally rather elongated, rod-shaped, frequently more or less bulbous os club-shaped at the ends. Some of these swollen cells arise by division of the swollen initial cells, but also the younger tips of the rods may become the seat of swellings. Division is now continued for some time on the

Fig. 47.
Actimonyces 1920.

medium, and at the time when development is about to come to a close in the given area of the medium, the longer rods begin to divide into shorter and shorter segments, which often remain in their position without being displaced to each other. This gives rise to the banded or striped forms we saw in the branched fungi, and with which we are familiar in the diphtheria bacillus. Or, the small segments are displaced in their position to one another, showing the same angular formation as we know from the longer forms. Growth continues for a very long period on the media, and in older colonies we shall constantly find, in the periphery, long threads and rods which continue to grow and, in the course of some time, become the subject of segmentation. The margin of such an older colony presents a picture bearing the most striking resemblance to the one we know from those ray fungi whose mycelium divides (Cp. Fig. 48) into segments.

In appears, thus, that certain branched fungi may show transition to a different mode of growth, the »angular« growth, so that we have now one, now the other mode of reproduction, without it being possible to say anything definite as to the underlying cause of this phenomenon. As far as the fungus last dealt with is concerned, the growth could not be examined at the stage when is was more richly branching, but, I am fortunate enough to possess a fungus which shows a quite parallel transformation.

This fungus was discovered and obtained in pure culture from a swab from a soldier. These swabbings undertaken with a batch of mariners, revealed the curious fact that ray fungi, belonging to group II b, are of quite common occurrence on the tonsils and in the naso-pharynx of healthy persons. Only in a few cases did I succeed in obtaining pure cultures from the colonies found, and this for several reasons. In the first place, these colonies grew at an extremely slow rate in the first generation on the culture medium

Fig. 48.
Actinomyces 1920. Cultivated on agar at room tp. 1 month old. High power dry lenses × 700.

employed, which was agar with addition of ascitic fluid and dissolved hæmoglobin. The largest colonies of the first generation could only with extreme difficulty be discerned with the naked eye, while they could be readily distinguished under low power.

Another difficulty in sowing from the small colonies was that they adhere rather strongly to the medium, and, considering besides, that closely adjacent neighbour colonies of bacteria will, as a rule, further render the isolation of the organism it is desired to cultivate, difficult, since it must be undertaken under a magnification of about 50 times, beneath the microscope, it will not seem so strange that it was only in a minor part of the cases that pure cultures were obtained.

(It might no doubt be comparatively easy to devise a technique by which the difficulties would become reduced; primarily, a more scattered placing of the colonies should be aimed at).

These swabbings, which were undertaken midwinter — in distinction from the soldier swabbings mentioned under Group I, which were performed in the summer — did not produce growth of one single ray fungus of Group I, which seems to indicate that the spores of the latter saprophytes in summertime are conveyed about by the air and readily inhaled, while the chance of this is

much reduced in winter. (In the first series of swab-takings, I did not yet notice the small colonies of fungi belonging to Group II b. It is however reasonable to suppose that such have been present at that time too, but have escaped my notice.)

Even though I did not succeed in obtaining pure cultures from a very large number of the ray fungi found in these swabbings, it was easy to form a fairly good judgment of the frequency with which they occur.

In the figures stated below are included those colonies only which, partly by examination under low and high-power dry lenses, and partly under immersion lenses, displayed the typical arrangement of the ray fungus, with long branched filaments radiating in all directions, forming a sort of aureole round the colony. It proved that in no less than *18 out of 32 tonsillary swabs, and in 9 out of 100 naso-pharyngeal swabs, were these fungi found.*

The young colonies of these ray fungi are easily recognizable from young colonies of Group I fungi in that they are but slightly transparent at an early stage of their development, being usually of a dark brownish appearance when seen under the microscope, in distinction from Group I colonies, which remain clear and transparent for a long period.

The ray fungi of the present group which I succeeded in cultivating, all showed similar transformations as Actinomyces 1920, when cultivated on artifical media; most of them did already in the second generation pass into the bacillary shape with »angular« growth, so that these cultures bore no resemblance whatever to the mother colonies. In some few cases there was a more graduated transition, the which, being totally parallel with the transformation found in Actinomyces 1920, we shall follow a little closer.

ACTINOMYCES 179.

In the first generation it grew like a typical ray fungus (see Fig. 49) with long, branching, slightly club-shaped, filaments sprouting in all directions. Second generation showed, on ascitic agar, to begin with a quite similar appearance. Rate of growth extremely slow. After 5 to 6 days' growth the first septa began to appear, and, examining the colonies after a few more days' growth, they exhibited very slight resemblance to the picture seen a few days earlier. Only in the marginal portion of the colonies were found some isolated threads, while the main part of the colony consisted of rod-

shaped segments, displaced in their position to each other so as to form the most different angles.

The colonies, which at the beginning adhered strongly to the medium, were now of a somewhat softer consistence, it being now much easier to inoculate subcultures from them.

Third generation grown on ascitic agar and ordinary broth agar formed a mycelium in rare cases, only, and then a very small one, the growth continuing immediately as »angular growth«, the fun-

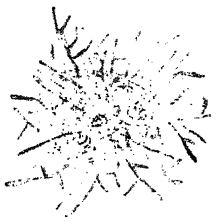


Fig. 49.
Actinomyces 179. Ascitic hæmoglobin agar. Cultivated 2 days at 37 ° and 3 days at room tp. High power dry lenses × 700.

gus developing now at a much more rapid rate than in the previous generations. The fungus has preserved this appearance ever since. In young cultures the segments are somewhat longer than in older ones; in old cultures the colonies consist exclusively of small short rods and spherical elements which are attached to each other to a greater extent than was the case with the elements of Actinomyces.

In broth the fungi grew at the bottom like small scales, to begin with at a very slow rate, but already in the first subculture did the growth become more abundant, the broth showing a sort of turbidity which however differed from the diffuse cloudy growth we know from many bacteria. Here, it is quite distinctly due to minute isolated granules which, in the course of a few days, settle on the bottom and leave the broth perfectly clear; concurrently, the first small scales appear on the surface. In later subcultures, surface growth constantly occurs at an early stage.

This transformation in respect of morphology, which we have now traced step by step in all points, is in perfect accordance with what was found in Actinomyces 1920, the two modes of growth: ramifications and »angular growth« occurring side by side just as

in the named fungus. We also meet the same polymorphism; the same club-shapes, the same banded forms, and the same small final cells.

We have here again step by step followed the transformation of a fungus which initially presented the typical picture af the ray fungus and later assumed a character according to which it would be classified among the corynebacteria.

By examination in moist chamber of hanging-drop of broth preparation of Actinomyces 1920, I succeeded in demonstrating — in the work previously mentioned — that the individual segments of the «banded« elements frequently grow out transversely on the longitudinal axis of the initial element, so as to form a sort of side branches, or, in case all the segments germinate, a series of bacillary bodies situated side by side; quite analogous transverse outgrowths are, as has been shown, exhibited by Nocardia farcinica, and similar relations may moreover be encountered in several of the fungi described in the above. Just as commonly, however, will the individual segments within such a »banded« element be displaced in their position towards each other, simultaneously with their displaying some swelling.

Streptothrix canis (Kràl).

Being unaware of the above described transformation within the ray fungi — from fungi which multiply by the formation of side-branches, to fungi which show only a sparse ramification whereas they display the mentioned »angular growth«, — the idea that Streptothrix canis should be related to the ray fungi, would not occur to one.

On most media it forms citron-coloured colonies of a pasty consistence, easily detachable from the medium. In broth a scaly surface growth appears early, spreading to a pellicle from which small flakes readily sediment through the clear fluid. The morphology is quite analogus to that of Actinomyces 1920, described after this fungus having passed into the stage of »angular growth«, only, the individual elements in Streptothrix canis are somewhat shorter and more slender. The polymorphism is quite identical; we meet the same »banded« appearance, the same clubs and the same short final cells. Branched forms occur, but very rarely. As in above named fungi, »angular growth« is a constant feature (see Fig. 50). Is is probable that, at the time when the name Streptothrix

was given to this fungus, it has displayed a more branching growth in the cultures than it does at present.

On reviewing the literature extant, it will be found that similar transformations have, no doubt, been observed by earlier investigators, where the objects under observation were doubtless true ray fungi. Thus *Bruns* found that a fungus, which originally had an elongated branching filament, later passed into a more bacillary mode of growth on artificial media. (As for the descriptions of these

Fig. 50.
Streptothrix canis. Cultured on agar at 37° for 16 hours. Immersion lenses × 1000.

transformations obtaines, that is seldom transpires from the works of what nature this »transformation« in reality is; whether it is merely a richly branching fungus that divides into segments, that is to say a fungus with mycelial formation, or whether a transformation to »angular growth« has actually taken place).

Abramow made similar observations and says: »Aber schon die 2 Generation degenerierte gewöhnlich zu Stäbchenform«.

Dresel likewise demonstrated that a ray fungus, which was found in the shape of long branching filaments in the pus from which it was isolated, afterwards grew in the cultures as rods.

Plaut states that three different anaerobic ray fungi, in the original preparations from the pathological material, were long branching filaments, while in the subcultures, they grew as short, usually non branching, bacilli.

For one of them applied, that in the first generation it grew as elongated branching threads. Both in these and other works, when the question is of the short forms in smear preparations, a very marked polymorphism is constantly stated to occur, and, at the same time, the various authors often make use of the resemblance of these preparations with similar ones from cultures of bacillus diphtheria in order to convey to the reader the impression of both morphology and arrangement, the accompanying illu-

strations frequently showing most beautifully the arrangement in Y or V shapes, a manifestation of the »angular« mode of growth of the cultures.

A question which early arises, is: should the final cells be conceived as spores in the same sense as previously defined? The answer must be in the negative!

These cells lack the uniformity which was found in Group I fungi, and they also lack the augmented resistance to exogenous deleterious influences; I have at any rate not succeeded in demonstrating any difference in resistance against heating and dessication between the undivided mycelium and the final cells. Both the mycelium and the final cells absolutely resist dessication for at least seven months. Whereas 6 strains of Bacillus Coli, of the Proteus group, and of Staphylococci, chosen at random for comparison, under identical conditions resisted dessication for six to seven weeks only, at most. As for thermal resistance, it proves to be less than for the spores found in Group I fungi.

Another feature by which the final cells are distinct from the spores, is that they germinate under the same conditions under which they have been formed. The aerial spores of Group I fail to do so, and, in those cases in which I examined this relation in regard to the true bacterial endospores, I did not find this power of germination in them. Spore formation was also here a manifestation of the fact that further development is checked within the given area of the medium.

The various final cells may possibly differ, but such difference would no doubt be extremely difficult of demonstration.

In my opinion, the designation spores should not be attached to these segments.

On examining the accessible literature with the object of finding descriptions of fungi resembling those here described under Group II, several papers will be found, — besides those already mentioned — in which fungi are described which are morphologically identical to those here dealt with.

The descriptions are however often so defective that it is difficult or impossible to form any clear judgement as to which fungi we are concerned with.

We have already mentioned *Nocard's* and *Vallée's* works on Nocardia farcinica and Streptothrix polychromogenes, respectively.

Both these authors are a little bewildered as to the nature of

the various elements; *Nocard*, for instance, erroneously talks of endospores in Nocardia farcinica, and *Vallée* of oospores in Streptothrix polychromogenes, inasmuch as he conceived the segmentation resulting from the filaments as a process analogous with the one demonstrated by *Sauvageau & Radais* in the ray fungi of Group I.

Eppinger is one of the first to give a good description of a ray fungus belonging to Group II. He correctly describes the primary mycelium and the segmentation into smaller elements. Strange enough, he conceived the ramification as false, and gave the name Cladothrix asteroides to his fungus, notwithstanding the fact that his illustrations show with all desirable distinctness that the branching is true enough. *Eppinger* found, that the small elements possessed the power of spontaneous motility, a finding which later investigators have not been able to corroborate.

Furthermore, *Nocard* described a microbe obtained in pure culture from a case of ulcerous lymphangitis, which morphologically as well as culturally, belongs here. Here, as often before, *Nocard* points out the similarity with diphtheria bacilli, and also the marked polymorphism.

Feistmantel subjected Nocardia farcinica to investigation, finding in young cultures, long threads, in older ones, short segments.

Silberschmidt describes a Streptothrix caprae, which likewise belongs here. He found also long threads in young colonies, and rods in older colonies. »Cette transformation en batonnets s'observe dans toutes les cultures superficielles«. He, too, emphasizes the very marked polymorphism.

In several other works, *Silberschmidt* rightly points out that direct microscopy of pathological products does, doubtless, not suffice for determining the nature of the pathogenic fungus, and he believes, no doubt correctly too, that different fungi of a different nature in many cases go under the same designation. Like other authors who have studied the ray fungi closely, he emphasizes the importance of being clear upon the pronounced polymorphism of the fungi.

Gruber also describes a ray fungus which formed a small mycelium, only, before dividing into segments. (Gruber calls these segments, fragments).

The description of the appearance of the cultures and the morphology of the fungi, corresponds in all essentials to the picture presented by Group II b.

Casabo found in his own sputum a richly branching fungus which, after some days' growth, divided into short elements. The appearance of the colonies was in every respect typical.

Schabad beautifully describes a ray fungus which forms aerial hyphae, and which no doubt belongs here. It is a little difficult to get a clear impression of the short cells that occur in somewhat older cultures, but, according to the description of the appearance of the cultures, there can be no doubt but that the described fungus belongs to Group II.

Litten & Levy obtained pure cultures of a ray fungus which, in similarity with Actinomyces 179, exhibited a slightly »turbid« growth in broth in the first generation, to grow later in same medium without turbidity, forming at an early stage a pellicle on the surface of the broth, from which small flakes readily sedimented. The morphology corresponded perfectly with that of Actinomyces 179. *L. & L.* do not feel quite convinced that the short forms are derived from the long threads in the pure cultures; they are however most inclined to believe that they are.

The pronounced polymorphism is described here too.

Gjorgjevic found no less than four ray fungi as the causative agents of various affections, showing perfect cerrespondance with one another physiologically as well as morphologically (it is however an open question whether the methods of investigation applied to elucidate the physiology of the fungi have been sufficient to determine the identity of these four fungi). The morphology of the colonies distinctly shows that the fungi concerned belonged to Group II.

In conclusion we shall just refer to *Lieske's* work on the ray fungi. As previously mentioned, he unites all ray fungi into one single large common group, which he divides into subgroups according as the fungi form long threads, medium-sized threads, or short threads. It is not necessary further to point out that this mode of classification is but slightly adequate. For, we have seen, for instance, how a fungus may have long filaments in the first generation, and in later generations no threads at all. Strange enough, *Lieske* has apparently not quite realized the essential difference which the different ray fungi present in respect of *spontaneous segmentation* of the substratum mycelium. In his extensive and in many points very valuable studies on the ray fungi, it has of course not escaped his notice that some fungi broke down

into short elements, but, strange enough, he interpreted this phenomenon as a peculiar delicacy on the part of the threads of these fungi, after having first correctly demonstrated — in a hanging-drop of broth preparation — that these rod-shaped elements germinate into a unicellular mycelium just as the spores of Group I fungi. That this mycelium later divides into segments by means of septa, he has however not expressly pointed out.

Not until I was on the point of bringing this work to a conclusion, did I succeed in obtaining an anaerobic strain of actinomyces, pathogenic to man. In the pus sent in to the Serum Institute for examination it appeared as long, thin, branching, threads, which were not furnished with typical club-shaped capsules.

Pure cultivation succeeded fairly easily, the fungus showing an appearance in the cultures which in all essentials corresponded to the previously described, so that we need not enter further into details about this appearance.

Aerobically the fungus failed to grow at all.

In order to examine it according to the methods otherwise employed in the present work, it was sown on broth agar in Petri dishes, suitably sized cubes of the inoculated agar plate being afterwards excised and placed in test-tubes that were furnished with *Buchner's* well known stopper for anaerobic cultivation.

The growth was then studied in the usual manner, the cubes being placed on slides after varying periods of incubation.

The pictures appearing showed that this fungus belonged to Group II b. Primarily it forms an asteriated branching mycelium which, after varying periods, divides into segements that become displaced in their position towards each other, just as has been described in regard to the fungi previously dealt with; and here — as in certain of the preceding fungi — we encounter the peculiar feature that colonies, which early show the typical »angular« arrangement of the elements, grow side by side with colonies that do not divide into rod-shaped elements until at a much later stage.

This feature is likewise beautifully seen, when a liberal amount of culture be disseminated in fluid broth agar which is afterwards allowed to congeal. A dense growth of colonies will now appear

in the agar from about 1 cc. below the surface downwards towards the bottom of the tube, and, having removed the agar cylinder from the tube, it is fairly easy to cut thin discs out of the same and subject these to microscopic examination. There will always be easily accessible colonies lying just beneath the cut surface and which have thus retained their natural shape. In this way it proved easy to obtain a general survey of the arrangement of the elements, just as by *Harbitz & Grøndal's* method with the preparation of frozen sections of the cultures, by which these observers obtained beautiful pictures of the arrangement of the elements within the colonies.

By this method, too, it is distinctly seen that some of the colonies are more compact and finished of appearance, giving out but a few minute lateral outgrowths, while others are more loosely formed with long branching threads radiating in all directions.

The fungus here described forms no aerial hyphae.

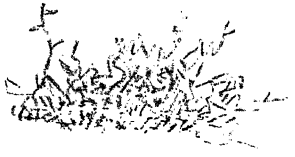


Fig. 51.
Actinomyces hominis. Cultivated in broth-agar, 5 days at 37°. Margin of colony. × 700.

Fig. 52.
Actinomyces hominis. Broth agar, 5 days at 37°, Margin of colony. × 1000.

It was evident, from earlier works already, that the systematic position of the anaerobic Actinomyces hominis must be in Group II b.

Wolff & Israel already demonstrated that the fungus, which had a long-threaded growth in the affected organism, appeared essentially as short rods in the cultures. By inoculating the fungus in eggs, only, did they procure a growth of long branching filaments. Later, *Wright* has beautifully demonstrated that the fungus grows also in the cultures as long threads, which however divide into short segments, a character which of course has the effect of producing — in smear preparations — a picture suggestive of a culture of rods. Therefore, in order to show that the fungus

forms a mycelium in the cultures also, he had to cut the colonies into sections on the microtome to avoid disturbing the arrangement of the elements in the cultures. *Shiota* and *Harbitz & Grøndahl* arrived at similar results. In all these works, the similarity of the segments with diphtheria bacilli is referred to, and also the arrangement at different angles to one another of the elements in smear and suspension preparations. *Silberschmidt* (1901) gives a description of the morphology, which I shall quote because it quite briefly produces a picture corresponding exactly to the one we know from Group II b.

»Der Pleomorphismus ist sowohl in Bezug auf Form als auf Färbbarkeit ein sehr grosser. Die Fäden erscheinen oft wie aus Stäbchen zusammengesetzt, namentlich in den Präparaten nach *Gram*. Die kurzen Formen sind meist gebogen an den Enden entweder zugespitzt oder kolbig verdickt, parallel oder in Winkelstellung angeordnet, ziemlich oft verzweigt, Diphtheriebacillen ähnlich.

Auffallend war auch, dass die Stäbchen und Fäden nicht alle gleich dick erschienen, und dass die verschiedenen hier angeführten Formen in einem und demselben Präparat neben einander beobachtet werden konnten.«

It does not distinctly appear from the various works here cited, how far a transformation to »angular growth« occurs in certain cases. In the previously named work by Plaut is stated that certain anaerobic ray fungi display this mode of growth.

CORYNEBACTERIA AND MYCOBACTERIA*)

For most of the bacteria included under above designations obtains that they frequently exhibit true branching in the cultures.

Metchnikoff and *Klein*, who were the first to demonstrate this mode of growth in tubercle bacilli and diphtheria bacilli, respectively, attached great importance to this feature for classification, advancing as their opinion that the microbes in question could henceforth not be included in the group of true bacteria, but should be classed with the morphologically higher differentiated fungi.

In the following years quite similar ramifications were detected by other investigators, not only among the named bacteria, but also in numerous other microbes belonging to widely differing groups in the world of bacteria.

The valuation of these findings was greatly at variance. Some bacteriologists agreed with *Metchnikoff* and *Klein*, others held that there was no reason to attach any special importance to these often relatively rarely occurring branching forms, and more especially the fact that numerous strains of one bacterial species whose members might occasionally exhibit branching growth, never did present such side branches, resulted in these ramifications being interpreted by many — indeed, we dare say by most — investigators, as involution forms, which was taken to indicate that they should be due to external deleterious influences to which some strains were susceptible, others not. However, branching mode of growth was not the only morphological feature by which coryne- and mycobacteria bore resemblance to other microorganisms whose position falls outside the group of true bacteria in the botanical system.

*) I use the terms suggested by Lehmann & Neumann.

In the preceding section we have thus again and again found occasion to refer to the resemblance between ray fungi, and, for instance, diphtheria bacilli. And, to this comes, moreover, that some of the microorganisms known under the name of Mycobacteria occasionally grow within the animal organism in quite the same manner as certain ray fungi (compare section below on growth-forms in the animal organism).

That the isolated finding of *some few* genuine side branches in a microorganism is insufficient ground for excluding same from the group of true bacteria, I realized at an early stage of my work; such findings are far too common to justify that. I would be apt to believe that careful direct microscopy of the cultures would occasionally disclose a few side branches in every bacterial species.

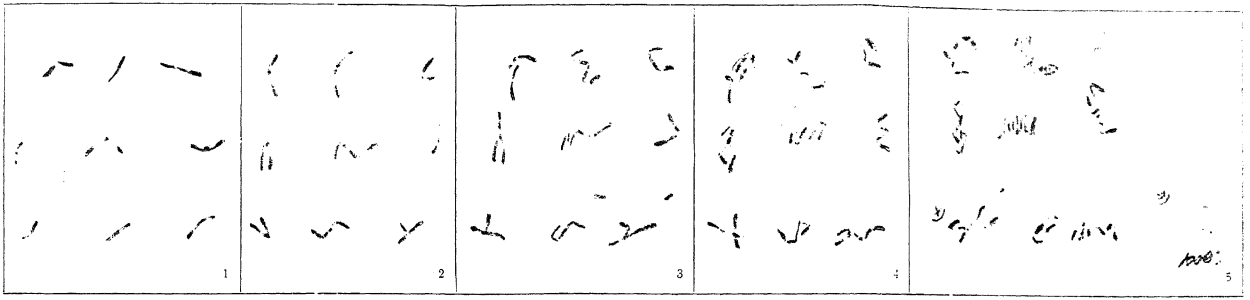
As it is, there are however other morphological characters, besides a branching growth, in certain of the microbes with which we are going to deal in the following, which are of much more constant occurrence than the side branches, characters that distinguish them sharply from other groups of bacteria, which never exhibit same.

Now, in order to find out what points of resemblance and what points of difference there are between the previously described fungi, — to which the corynebacteria and mycobacteria are most closely related, — and these microorganisms, I have subjected the latter also to investigation, hoping that I might possibly be able to contribute however slightly to throw some light on the problem of the systematic position of these microbes, in as far as the old controversy concerning these relations can at all be settled by morphological investigations.

Although most of the microbes dealt with in the following sections have been excellently studied before, and many of them, for instance the diphtheria bacillus, with such thoroughness that there can be but little to add, I propose here, as in the preceding sections, to begin by describing the morphology of the various bacteria such as it appeared to me, and afterwards compare my findings with the descriptions found in earlier works.

Corynebacterium diphtheriae.

For these investigations I have employed, partly the diphtheria bacilli coming under my hands under the routine diagnostic work at the laboratory, partly twenty strains most kindly submitted to



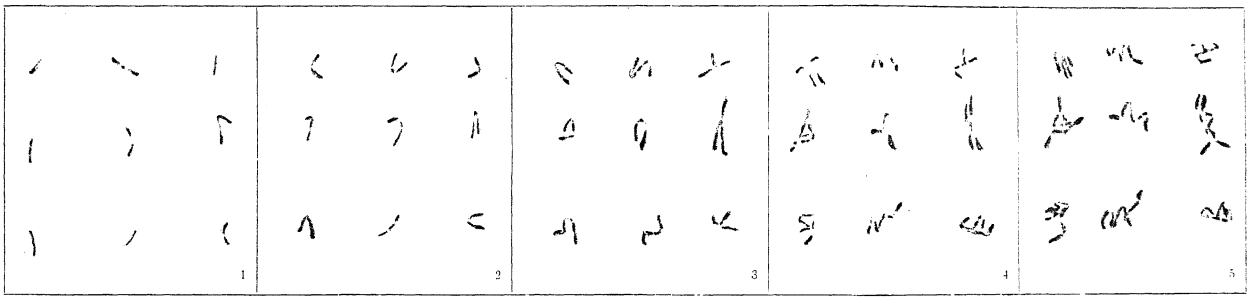


Fig. 53. Corynebacterium diphteriae.

me by Dr. *Max Christiansen*, who has just completed an extensive work on various physiological and immunobiological phenomena in diphtheria bacilli, so that these 20 strains have been closely investigated in regard to all their physiological reactions.

Examining young cultures of these numerous various strains of diphtheria bacilli, the same picture constantly appears, namely the one we know from certain of the fungi belonging to Group II b, that is to say those which have passed into the mode of growth designated as »angular«.

In quite young colonies the individual elements are constantly arranged so as to form different angles on each other and, inquiring further into the constancy of this phenomenon, we find that it is absolute.

In case an element lies isolated on the plate, that is to say is not hampered in its free movements, the individual segments will constantly be seen, subsequent to division, to be displaced in their position towards each other, just as we saw it in the ray fungi. The larger the colonies grow, the more do the individual elements become hampered in their free mobility by neighbour elements, but it is nevertheless quite easy to follow the course of development for some lenght of time and to see that it is constantly the same. When the colonies have grown to such a size as to become less transparent, the »angular« formation can be followed in the marginal portion only, where free elements will nearly always protrude. Fig. 53 shows the angular arrangement of the elements in a young colony followed as long as the image stands out fairly sharply under high-power dry lenses. This »angular« mode of growth is an absolutely constant phenomenon in all the strains examined.

Now, on looking a little closer upon the individual elements, we shall also here find a beautiful accordance with the morphology of those ray fungi which exhibited »angular« growth.

Taking, for instance, as inoculum elements from a culture in which development has practically ceased, we shall find a mixture of short and long forms of widely varying shape, from minute coccus like forms, through somewhat more elongated, often club-shaped, bacillary forms, to quite long, frequently transversely banded elements which, just as in the case of certain ray fungi, must be interpreted as resulting from segmentation of long rods.

Many of the inoculated longer forms are but slightly refractive,

with a markedly granular protoplasm, whereas many of the shorter forms are strikingly highly refractive.

Following the development further, we shall see that the short isolated forms and certain of the segments of the long banded elements start to grow on the fresh medium, while the great majority of the granulated rods take no part in the development. The small coccoid elements usually swell more or less before giving out one or two, rarely more, small tapering sprouts, by which arise the bulbous. club-shaped or fusiform, usually rather short elements which we know so well from young cultures of diphtheria bacilli. The fusiform element having attained a certain size, a division line, dividing it in two, generally arises approximately in the middle of the element. After the lapse of some time these two parts are pushed from one another, not completely however, as they remain attached at a small area on one side, so small as to be manifested only by the continued attachment of the elements. This displacement usually occurs slowly, occasionally however more like a sudden »snapping«, as *Hill* has it.

Concurrently, the proximal ends of the newly formed rods assume a rounded shape, and we have thus two small short club-shaped coherent rods. The relative displacement of the elements is doubtless due to this swelling of the proximal ends of the rods, by which a mutual pressure is exerted. The next thing that happens is that these elements elongate somewhat, may be at both ends, but most commonly at the proximal end only. In case only one »leg« of the angle grows, we get pictures suggestive of the letter Y, in case both »legs« grow, we get an H-shaped picture. These new elements again grow, dividing in a similar manner after some time's longitudinal growth, after which the new-formed rods become displaced in quite the same manner as described above. The pictures resulting from this mode of growth are suggestive of the letters of the Chineese alphabet.

In these young cultures a marked polymorphism may be said to be a constant feature; albeit the individual elements are usually fairly uniform in size, there will always in among the short rod forms be found some long, frequently club-shaped, elements, which often at an early stage divide into short segments. These segments continue to grow on the medium after more or less swelling and under mutual displacement. Occasionally we encounter a transverse growth similar to that previously described in certain ray fungi.

Besides these forms, nearly all cultures display some genuinely branched forms which appear immediately from the initial stage of colony formation.

In some strains branching forms are rather common, in others they are extremely infrequent of occurrence. These true branching forms may be encountered at every stage of development and on every culture medium, it being not possible to state anything definite as to the conditions of their occurrence. For instance, a sixteen-hour broth culture of the well-known American Bacillus diphtheriæ, *Park & Williams*, which is employed all over the world for toxin production, exhibited short genuine side-branches in such numbers as to be an all prepondering characteristic of the elements; including such branches only as are not separated from the main stem by septa of any kind whatever. In next subculture on same medium, under apparently identical conditions of growth, only very few branches were found.

By direct microscopy of the cultures (in order to distinctly perceive the septa, it is necessary to apply high power immersion lenses) we find, which is obvious, much more easily a far greater number of true side branches than in the suspension and smear preparations in which, partly, it is difficult to decide how far the branching is genuine or only due to opposition, and, partly, the side branches are frequently detached by these procedures.

(If the question is to examine larger colonies) it may prove practical carefully to spread the colony all over the inoculated surface by means of the inoculation needle. By this means we shall often obtain a very beautiful general impression of the morphology).

The older the cultures, the larger the number of long club-shaped, frequently segmented, forms on the media; but, at the same time, there is an increase in the number of quite short forms (it must be remembered that the long segmented forms, too, consist of small elements) while the number of medium-sized rod forms is diminishing. By subcultivation on a fresh medium, the course of development remains the same. As for the long segmented forms, it is most commonly only part of the segments that germinate subsequent to swelling, while other segments, usually slightly refractive ones, do not take part in the development. The minute coccoid forms sprout, as already described, while the great majority of the rod-shaped elements fail to take any part in the development, and, the older the culture, the more conspicuous does this feature become.

It will appear from above description that the picture is extremely polymorphous all through the phases of development, and I do not see that we have any foundation for appointing any one of the forms described, as being the »normal« one.

Polymorphism is here, just as in the ray fungi, a typical feature.

Fig. 54 represents a three days' culture on ascitic agar. — So far in regard to the external form. As for the internal structure of the bacteria, we have already mentioned the septa. Whether these arise in a similar way as seen in certain ray fungi, initiating peri-

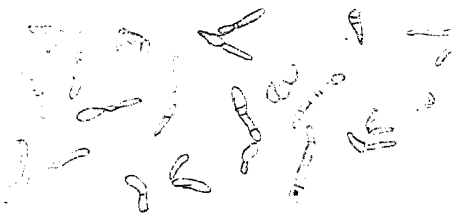


Fig. 54.
Bac. diphteriae. 3 days culture on ascitic agar, at 37° × 1000.

pherally, and whether the angular mode of growth in Bac. diphtheriae should thus be due to similar relations as those supposedly obtaining in the ray fungi I have not succeeded in directly demonstrating, byt I would think that there is good reason to believe that it is so.

At a fairly early stage of development, the so-called metachromatic granules appear, generally discernable with equal distinctness in unstained as in stained preparations; unstained they present themselves as well defined, round, highly refractive bodies. The picture is perfectly analogous with the one they exhibit in stained films, and supposedly they consist in specially highly concentrated protoplasmic areas. I have not been able to establish any correlation between these granules and the development of the fungus.

Very frequently such a granule is found in the middle of a rod-shaped element, at the very place of division, and it often looks as if the granule were parted into two in the process of division, but division occurs equally well without the occurrence of such granules.

With advancing age, the protoplasm in numerous rods grows less refractive, with scattered fine granules, frequently the membrane only of the cell is distinctly visible. Such elements will sel-

dom grow when transplanted to fresh medium, if they do grow, a »reparation process« takes place primarily, the cells regaining gradually their refractivity, their protoplasm becoming homogenous without granules, after which they start to grow.

The elongated transversely banded forms, which occur in very varying numbers in the different strains, often prove to consist of highly refractive and slightly refractive segments, alternately. By transfer to fresh medium, the slightly refractive elements usually fail to take any part in the development, as already referred to. The cultures frequently present some enormous swollen forms. In transplantations these occasionally do grow, occasionally do not. As for the quite short forms, partly the isolated club shapes, partly the small segments of the elongated transversely banded forms, there is no more reason for interpreting any of these as spores, than there was in case of the cells designated as the final cells in the ray fungi. Using for inoculum for instance a relatively young culture in which these forms abound, most of them will grow on the fresh medium, but as soon as the culture from which they are sown is somewhat older, only a minor part of them germinate.

The different strains of diphtheria bacilli may vary a great deal morphologically. In some the short, rather plump forms predominate, while in others the elements are slender and more elongated. I have not been able to establish any parallelism between the morphological and physiological variations.

In broth the morphology is broadly speaking the same as on solid media. In very old cultures, quite short, almost coccus like elements preponderate. In subcultures, as in cultures on solid media, the short forms possess preeminently the power of germination.

During these investigations into the diphtheria bacilli, my attention was arrested by a peculiar substance formed by certain strains when growing on serum containing media, such as ascitic agar and Löffler's serum.

After varying periods of growth, — sometimes immediately from the start, — these strains form some *highly refractive* granules located on the outside of the cells; often several granules on one cell. At the outset, these granules are quite small and spherical of shape, but later they increase in size and become irregular of shape. They are very easily detached from the bacilli. They stain very badly and are not acid-fast. They are partly dissoluble in absolute

alcohol. They have no doubt been noticed earlier, but have probably been interpreted as swollen diphtheria bacilli.

These granules do not participate in the true development of the microorganism, as they fail to germinate when transplanted to fresh medium. I have not succeeded in elucidating their nature and significance.

The most highly toxic strains frequently formed these small bodies in abundant numbers; they did however also occasionally abound in atoxic strains.

Now, on looking back upon the morphology of the diphtheria bacillus, we find the finest accordance between this microbe and the various ray fungi, more especially with those which have passed into the »angular« mode of growth.

We find the same, usually sparse, growth of side branches, the same polymorphism, the same club-shaped and fusiform elements, the same transversely banded elements and final round cells. And, moreover, the previously described transition, found in true ray fungi, to microbes exhibiting the »angular« mode of growth, which — the transition having been accomplished — bears the most striking resemblance to the mode of growth displayed by Bacillus diphtheriæ, and the which is otherwise encountered in no other bacterial species (apart from those to be mentioned in the following), — all this seems in my opinion to speak strongly in favour of classing the diphtheria bacilli with the Ray Fungi.

Besides diphtheria bacilli, I have examined a series of corynebacteria isolated partly from sputa, partly from swabs taken from tonsils and naso-pharynx.

They are all of them Gram-positive, extremely polymorphous microbes, quite similar to the bacteria which pass under such names as Pseudodiphtheria bacillus, Bacillus Xerosis, and others.

Notwithstanding the differences displayed by these microbes, there are many points of similarity, and they all exhibit the »angular« mode of growth with absolute constancy.

One of these microorganisms showed especially beautifully the mentioned transverse banding, a few days old cultures on ascitic agar, incubated at 37°, containing exclusively long finely striated forms. See Fig. 55. The mentioned transversal mode of growth also appeared finely in subcultures. The older the cultures from which inculations were made, the smaller the proportion of individual segments that germinate on fresh medium, which again indicates

that these segments should not be interpreted as a sort of spores.

Branching forms occurred to a greater or less extent in all the cultures.

According to their morphology these microbes, too, must be reckoned with the ray fungi.

A description corresponding to the above given in regard to corynebacteria, may be found in most authors who have occupied themselves thoroughly with the morphology of the diphtheria ba-

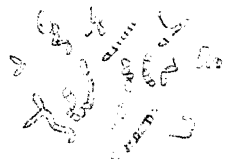


Fig. 55.
Corynebacterium 18. 48-hours' ascitic agar culture at 37°.
× 1000.

cillus, while there is great discrepancy of opinion in regard to the valuation of the polymorphism of these microbes.

Zarniko thus arrives at the conclusion that only the slender easily bent rods, are the normal forms, while all the other forms should be due to unfavourable environmental influences.

As mentioned before, *Klein* found a richly branching strain of diphtheria bacillus which, when cultured on artificial media, grew with considerably shorter forms than those found in the membranes. Injected subcutaneously into calves, it grew again into long branching filaments. This feature is suggestive of certain of the previously described ray fungi, which in the diseased tissues grew in long branching filaments, and in the cultures often as short rods.

Many other investigators have found richly branching strains, for instance *Fraenkel*, who points out that it is only a minor part of the strains that exhibit side branches, a fact that is not surprising in case it proves correct that the diphtheria bacillus is interrelated with the ray fungi.

Bernheimer & Folger found, like *Klein*, long branching filaments in the membranes, whereas the cultures displayed short forms only. By subcutaneous inoculation on guinea gips they recovered again long branching filaments, observations which also are in harmony with *Klein's*.

Lehmann & Neumann speak of strains »die ganz vorwiegend auffallend verzweigte Formen zeigten«.

Neisser and Ernst found similar morphology in the so called Bacillus Xerosis, and *Neisser* suggests that the parallelly arranged rods often seen in the cultures may have arisen by transversal growth of the individual segments in the transversely banded elements. He has not, however, directly followed the growth.

Scheller arrives at similar results as *Zarniko*, appointing the slender rods to be the normal forms.

Neisser & Gins are of opinion that the club-shaped diphtheria bacilli should be reckoned as the most typical elements of the cultures. »Das charakteristische Merkmal der äusseren Form des Diphtheriebacillus ist die Keulenform, die bereits Löffler erwähnt«. These authors correctly state that the club-shapes are to be found in quite young cultures also. »Ueberhaupt scheint die ausserordentliche Vielgestaltigkeit der Formen im mikroskopischen Bild für den echten Diphtheriebacillus geradezu typisch zu sein«.

Madsen correctly states that the coccus-like elements are predominating in old broth cultures.

In this connection we must refer to two works by *Spirig* and *Cache*, respectively, dealing with supposed diphtheria bacilli which were distinguished by especially profuse branching.

Spirig examined a microbe which he took to be a true diphtheria bacillus. It appears however with all desirable distinctness from the description, that the microorganism at issue must have been a ray fungus belonging to Group I. The morphology of the cultures both on solid and in liquid media corresponds in every detail.

It seems to be beyond doubt that *Spirig* has *not* worked with a pure culture of diphtheria bacilli.

And the same holds true of *Cache's* work. His cultures, too, must have been contaminated with ray fungi of Group I.

Abbot & Gildersleeve took upon themselves the task of examining whether the side branches of Bacillus diphtheriae arose under conditions that would seem especially adapted for producing abnormal forms. They found side-branching to be a quite common feature also in quite young cultures, but they are nevertheless inclined to interpret these forms as involution forms.

While above-mentioned authors found branching forms to be of frequent occurrence, there are others who either fail to mention

these forms at all, or who directly state never to have observed them.

When, for instance, *Przewoski*, in reference to branching, states never to have encountered true side branches in no less than 55 different strains examined by him, this must doubtless be assigned to a defective technique.

From nearly all these works it appears with great distinctness that branching forms are of frequent occurrence in some strains, while they are totally absent or only of scanty occurrence in other strains.

Now, as for the angular mode of growth? Did earlier authors find the same constancy of this feature?

In the available literature on Bacillus diphtheriae and related microorganisms, we find constantly references to this peculiar arrangement of the elements, and those authors who have directly followed the development, all notice it.

The first to demonstrate the angular mode of growth in Bacillus diphtheriae, was *Kruse*, who followed the development in hanging-drop of broth. He alleges never to have seen any other mode of growth.

Independently of the above named author, *Nakanishi* follows the angular mode of growth (1901). He just hints that there is the possibility that the individual segments of the long banded elements may grow out transversely on the longitudinal axis of the element.

Hill traced the angular mode of growth in »hanging block of agar« in moist chamber, arriving at the same result as above mentioned authors, demonstrating, besides, that certain other corynebacteria presented a similar mode of growth, while a very long series of other bacteria never exhibited these growth forms.

And, further, *Kurth* demonstrated the same.

In conclusion, I shall mention some more recent works by *Bergstrand*, partly on the morphology of Bacillus diphtheria, partly on a Corynebacterium discovered and cultivated in pure culture by this author.

As for the latter work, I must say that most of the author's illustrations bear very slight resemblance with the forms we are accustomed to meet in cultures of corynebacteria. Among other things, one of *Bergstrand's* pictures represents some long chains of large bacilli. I have never seen such forms and can fully join in

Nuttal & Graham Smith's categorical remark »Chains are never found«!

The first of the author's pictures resembles corynebacteria right enough, but in the later pictures the forms are extremely divergent from those commonly found, and, on studying the work more closely, we shall find a quite brief remark to the effect that the original Gram-positive microbe passed into a Gram-negative one in the course of a few generations; as it cannot be denied that some of the peculiar forms seen in the pictures are strikingly suggestive of the forms sometimes exhibited by certain Gram-negative rod-shaped bacilli, I find it difficult to discard the thought that a contamination of the original Gram-positive culture with a Gram-negative bacterium can have taken place. It is true that the author states to have performed the pure cultivation ad modum *Burri;* at what stage this pure cultivation took place, is however not expressly said.

Examining the morphology of Bacillus diphtheria, *Bergstrand* finds, quite correctly, that the banded elements consist of segments that should be interpreted as small cells, interrupted by areas which be believes to be filled with mucilage, or to be vacuoles with a liquid content.

Bergstrand also describes quite corretly that in these vacuole-like areas there are often to be found regular granules which frequently display lively molecular movements. *Bergstrand* calls them »Tanzkörper« and compares them to similar bodies found in certain yeasts. Any specific value cannot however be attributed to these granules, as quite similar granules may occasionally be found in bacteria which otherwise have no points of similarity with Bacillus diphtheriae; and as they are of quite common appearance in the so called Pseudodiphtheria bacilli. In a strain of these, cultivated in pure culture by the present author from a naso-pharyngeal swab, I thus found one isolated lively motile spherical granule in numerous of the short forms. Nor in this strain, did I succeed in demonstrating any independent power of growth of these isolated granules.

Bergstrand imagines the possibility that these granules may become free and grow on independently. I have many times tried to investigate this relation in cultures in which these granules were especially abundant. Categorically to deny that they may show independent growth would be preposterous, but we can say for certain that it is at any rate quite exceptionally, if ever, that

they grow when inoculated on the same kind of medium as the one on which they arose.

In the preceding investigations I have not included all the bacterial species reckoned by *Lehmann & Neumann* to the corynebacteria; these authors include, for instance, Bacillus mallei, and Becillus necroseos, in this group. I have not personally studied these microorganisms, but it appears distinctly from the literature that there are essential differences between these microbes and the above-mentioned corynebacteria; for instance the typical angular arrangement of the elements is never seen; nor do they display any transversely banded elements. The irregularities in form frequently presented for instance by Bac. mallei, do not either resemble those we know from the branched fungi dealt with above. On the whole, as far as branching is concerned, the statements differ very much. In regard to Bacillus necroseos, for instance, obtains, that many very careful investigators, *Bang, Schmorl* and *C. O. Jensen*, among others, have not been able to demonstrate any branching at all.

As for Bacillus mallei, there is no doubt that it sometimes exhibits branching forms; as previously stated, there are however numerous bacterial species which occasionally have this single feature in common with the ray fungi, but, one isolated seldom found morphological point of resemblance is decisively too unsafe a foundation for classification. Both these microbes however call for direct morphological investigation in order to get elucidated what points of similarity and what points of difference they present to the ray fungi.

Thus, as it appears partly from the literature extant, partly from our own experiences, there are among the so-called corynebacteria such as present many points of resemblance to the fungi which form mycelia, indeed so many points of resemblance that we cannot establish any definite boundaries between the latter and the former on a morphological basis. There is therefore every reason to place them into the same group within the botanical system.

The next thing that called for investigation was the morphological behaviour of the so-called Mycobacteria, when examined directly in the cultures.

Also as far as these microbes are concerned, a special position within the bacteria has been claimed by many investigators, while others class them with the ray fungi.

Mycobacteria.

Lehmann & Neumann include in this group the various types of tubercle bacilly, the leprosy bacillus, the smegma bacillus and moreover a number of acid-fast rod-shaped bacteria among which the best known is the so-called Bacillus of Timothy.

I have subjected this group to a similar investigation as the preceding ones, having first collected as great a number of the microorganisms as possible.*)

As for their morphology, it may be briefly stated that the microbes examined show great resemblance to certain ray fungi and to the corynebacteria.

Mycobacterium phlei. (Moeller).

(Institute of General Pathology, Copenhagen).

Inoculating from an older culture, we shall find on the surface

Fig. 56. Mycobacterium phlei. 18-hour culture on 3% glycerine agar, cultivated at room tp. Immersion lenses. × 1000.

Fig. 57. Mycobacterium phlei. Cultivated on 3 % sugar agar at 37° for 2 days. Immersion lenses × 1000.

Fig. 58. Mycobacterium phlei. 3 % glycerin agar. 3 weeks at room tp. High power dry lenses × 700.

of the medium chiefly short rods or almost spherical elements, which after the lapse of some time begin to give out one or more small sprouts concurrently with some swelling on the part of the initial cell, and we have a picture almost identical to the one we know from the microbes previously dealt with, the next stages of development showing likewise great similarity to the morphology of certain ray fungi. (See Fig 56).

Occasionally a small mycelium is actually seen to be formed, later dividing into segments, and the angular mode of growth will often set in at an early stage of development. In young cultures the individual segments are long, while they become shorter and shorter in older cultures. *The angular mode of growth is as constant*

*) I shall here no more than in the desciption of the corynebacteria enter into details as to the cultural or tinctorial relationships of these microorganisms. These relations have earlier been investigated so thoroughly that it would be superfluous to repeat them here.

a phenomenon here as it was in the preceding fungi, and true branching. (See figs.) *is formed often in great abundance especially in younger cultures.*

In old cultures in which development has practically ceased, we find the short, often oval, forms to be absolutely predominant. In this fungus there is also marked polymorphism, if however not so vigorously pronounced as in certain of the corynebacteria.

A Timothy bacillus from Staatsinstitut für experimentelle The-

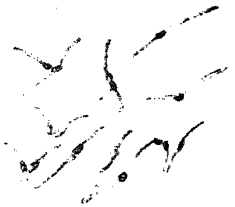


Fig. 59.
Rabinowitsch' Butter bacillus. 24 hour agar culture at 37°. Immersion lenses × 1000.

Fig. 60.
Bac. tuberculosis. (Frog) 5 days' agar culture at room tp. High power dry lenses × 700.

rapie behaved in quite the same manner as above described microbe.

Rabinowitsch' Butter bacillus (Staatsinstitut für experimentelle Therapie, Frankfurt a/M), shows, in young cultures, a picture quite similar to the one of the Timothy bacillus. See Fig. 59. It frequently appears as a *richly branching fungus, showing simultaneously the typical angular mode of growth.* It is polymorphous in the same sense as the preceding fungi. In the following we shall briefly describe some different strains of tubercle bacilli from cold-blooded animals.

All these, as however also the two preceding microbes, may be difficult to investigate in somewhat older cultures, because a fatty substance forms in the colonies between the individual elements, so that it is often only the margin of the colonies that is accessible to direct study.

Bacillus tuberculosis (from fish)
(Institute of General Pathology, Copenhagen)
is morphologically similar to the preceding microbes, only with less tendency to form long threads.

Bacillus tuberculosis (from blind-worm)
(Institute of General Pathology, Copenhagen).

Bacillus tuberculosis (from turtle)
(Staatsinstitut für experimentelle Therapie, Frankfurt a/M.).

The latter two microbes showed no divergencies from the morphology typical to the group.

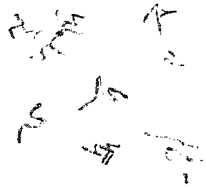


Fig. 61.
Bac. tuberculosis. Friedmann. 36-hour culture on 3% glycerine agar Incubated at 37°. High power dry lenses × 700.

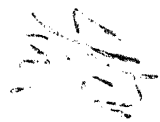


Fig. 62.
Bac. tuberculosis human, 20 days' culture on glycerine agar, at 37°. × 1000.

Bacillus tuberculosis (from frog)
(Staatsinstitut f. exper. Therapie) grows with genuine side branches and angular arrangement of the elements like preceding microbes. Colonies often round, with small branches protruding in all directions, the latter feature especially marked in young colonies. Often, however, the colonies are more elongated and sometimes, as first described by *Robert Koch* for the tubercle bacilli of warm-blooded animals, snake like, tortuous. See Fig. 60.

Bacillus tuberculosis. Friedmann
(Staatsinstitut f. expr. Therapie) likewise presents, in the gross outlines, an identical picture. Fig. 61.

I can only contribute slightly to elucidate the morphology of the different tubercle bacilli of the »warm-blooded« type. The strains I worked with all showed a very slow and sparse growth on the different media available, for the technique in use here, which of course impeded the tracing of the growth considerably.

Another difficulty is, that to obtain growth at all, it is necessary to disseminate the microbes very heavily on the media, and as only a minority will grow, the numerouh disseminated bacteria render observation very difficult.

With two strains, one of human origin, one aviary, I succeeded however in getting a fairly good impression of the morphology of some isolated young colonies on glycerine agar.

Both these strains showed marked angular arrangement, and presented true branching. Fig. 62 represents some elements from the margin of a small colony. We see both the true branches and the angular arrangement, and the slightly bulbous rods.

My experiences with tubercle bacilli of warm-blooded type are however so scanty that I shall not venture to draw definite conclusions from them; (one might of course here have had recourse to the old familiar methods of investigation, by means of which the morphology could be studied on media that are favourable to the growth of tubercle bacilli. As so numerous experiences have however been previously gathered that way, I prefer to apply to same). It seems however to be evident from the extensive literature dealing with this subject that there is every reason to believe that the »cold-blooded« and »warm-blooded« types of tubercle bacilli show identity in morphology in the gross features.

As for all the microbes dealt with in the above section applies that they do very often present protoplasmic granules within the rods and threads in highly varying numbers and arrangement. Neither as far as these fungi are concerned did I succeed in finding any independent viability on the part of these granules.

How far the angular mode of growth is an unchanging feature in the microbes described in the present work, cannot of course be decided, but, as seen in the numerous examinations performed, it always proved to be a feature of absolute constancy. There is however the possibility that the angular mode of growth might be superseded by a mode of growth undistinguishable from that exhibited by the true bacteria, of for instance the Coli-Typhoid group.

As we have already emphasized several times, genuine ramifications are by no means of infrequent occurrence in many bacteria which do otherwise bear no resemblance to the ray fungi; in some bacteria they are even encountered in great abundance. As an example I shall give a brief description of two microbes which are of some interest in this connection, following the excellent advice given by *Metchnikoff* in 1888 »il convient de ne pas se borner aux formes les plus habituelles et à cause de cela les mieux connues, il faut diriger les recherches sur des bactéries particulières, capables de donner quelques aperçus nouveaux sur la question de la morphologie du groupe en général.«

Rhizobium Luzerne (Institute of Agricultur and Veterinary Science, Copenhagen) one of the so-called Bacillus radicicola.

This microbe grows as round, clear, slimy, colonies on the media, and examining the colonies, true branching forms are frequently seen. Angular arrangement of the elements is however not seen. The individual elements are always placed freely in the colonies and will usually be seen to move lively about in same from the very outset of colony formation.

Except for the ramifications, this microbe presents however absolutely no points of resemblance to the morphology of the ray fungi.

Another microbe, cultivated from vaccine, bore greater resemblance to the ray fungi in the first generations on artificial medium. It formed yellowish-red, round colonies on most solid media; it was *Gram*-positive and grew well both at room temperature and in incubator at 37°.

The individual elements of this microbe were extremely small and delicate, in consequence of which it was difficult to follow the development.

The quite young colonies presented in the first generations a picture quite similar to the one we know from certain ray fungi with true branching forms, angular arrangement and marked polymorphism. As soon as the colonies had grown to a certain size, the angular mode of growth subsided, and the micro-organisms grew like non-branching bacteria without angular arrangement. At the same time they frequently displayed lively spontaneous motility.

After the growth of a few generations, there was nothing left to remind of the morphology of the ray fungi. This microbe however changed its morphology so rapidly that the information I succeeded in obtaining in regard to it was rather scant, and I have included it here only to draw the attention of possible future investigators towards similar findings.

I dare not either draw definite conclusions from this isolated finding in regard to a possible transformation from microbes which present the angular mode of growth to such in which this mode of growth is absent.

The papers extant dealing with the Mycobacteria, are so overwhelming in number, that we can here only refer to some few of them.

Metchnikoff was, as already mentioned, the first to demonstrate

richly branching and club-shaped forms in cultures of tubercle bacilli. (*Roux & Nocard* had however previously demonstrated quite small laterally placed knobs on tubercle bacilli in cultures). *Metchnikoff* interpreted the branching forms as atavistic throwback to branching progenitors.

Mafucci found similar branching forms in aviary tubercle bacilli (he assumes, no doubt correctly too, that the strain described by *Metchnikoff* must have been a tubercle bacillus of aviary origin, since its optimum temperature of growth ranged between 40 and 45°); *Mafucci's* cultures also frequently presented beautiful club-shapes. In his pictures some few of these appear to be transversely banded.

Fischel found branching and club-shaped elements in profuse numbers in Bacillus tuberculosis of human type, on account of which he classifies it with the ray fungi.

Babes and *Bruns* made similar observations. *Bruns* demonstrated that the same strain might show considerable variability in regard to ramification even though environmental conditions were apparently identical. (This finding is also in accordance with what we know from the ray fungi).

Coppen Jones also demonstrated branching forms, and emphasized the importance of very careful preparation. He detected the branching forms most easily in unstained preparations.

Lubinsky found no side branches, whereas he showed that certain strains of tubercle bacilli grew in long threads.

Marpmann found long branched filaments in sputa. (Under the roufine diagnostic work at the Serum Institute I have had opportunity to examine numerous sputa for tubercle bacilli and of cource had my attention focussed on the possible presence of branching forms. It is my impression that they are of rare occurrence in the usual smear preparations. In a single case, however, I found them in fairly large numbers. In this preparation the tubercle bacilli occurred simultaneously as short and more elongated rods).

Lubarsch also drew attention to the ramifications and polymorphism in Bacillus tuberculosis avium.

Fontes conceives the various granular bodies as being the centres of vitality in the cells, and he claims to have demonstrated their power of growing both in vitro and after inoculation into animals. It is however impossible to obtain a clear impression as to whether these granules are all of them identical with the intra-

cellular granules, or whether some of them may have been short segments.

The same holds true of the so-called *Much's* granules which, as is well known, were described by *Much* as being Gram-positive larger or smaller spherical non-acid-fast bodies.

Further, *Sanfelice* reports to have transformed a strongly branching microbe, perfectly resembling a ray fungus, into a microbe which was by no means distinguishable from Bacillus tuberculosis humanus.

We are indebted to *Vaudremer* for a very interesting work in which he demonstrated some strains of tubercle bacilli which lost their acid-fastness when grown on media to which no serum or glycerine had been added, to regain same when transplanted again to serum or glycerine-containing media. As for the morphology of these non-acid-fast forms, *Vaudremer* states that they often formed an asteriated mycelium, very similar to the one we know from the ray fungi. (*Vaudremer* has been kind enough to submit one of these strains to me, but unfortunately I did not succeed in making it grow).

Moeller, Korn and *Mayer* found various acid-fast rods resembling tubercles, which morphologically presented a picture identical to the one found by myself in similar investigations.

Korn for instance showed, in perfect accordance with my own findings, that a Butter bacillus detected by him and presenting the usual ramifications and club-shapes, consisted in quite young cultures of long threads and rods, to appear in somewhat older cultures as staphylococcus-like forms.

As for the various microbes isolated in pure cultures from cases of leprosy, there is a striking agreement between many of the records extant; and as far as many of the publications are concerned obtained that it is easy to recognize the nature of the fungi from the descriptions given, provided one is familiar with the morphology of certain ray fungi.

Thus, in *Levy's* work on the culture of Bacillus lepræ, there can be no doubt but that the microorganisms at issue have been identical with the fungi here described in Group II. Both as far as morphological and cultural relations are concerned, do they fit in with that group; and the same holds true of the microbes cultivated by *Babes*.

Kedrowsky likewise describes his cultures as containing long truly branching filaments, with some club-shapes, and septa forma-

tion; just the picture with which we are familiar from the ray fungi.

The microbes cultivated by *Czaplewsky* are evidently also ray fungi.

It appears, too, from *Barannikow's* work, that the polymorphism described corresponds with that of the ray fungi.

In alle these works the similarity with diphtheria bacilli is frequently referred to, just as statements are commonly encountered as to the arrangement of the elements in V or Y shapes.

From these works as well as from my own experiences it is evident that many of the so-called Mycobacteria cannot be differentiated from certain ray fungi for classification into the botanical system; and, when to this be added that, as far as many of them are concerned, it has been demonstrated (see section below), that they are able to form a well developed mycelium in the animal organism, there must be said to be very good foundation for classifying these microbes with the ray fungi.

GROUP III.

Streptothrix Chalceae (Kràl).

This fungus occupies a special position among the ray fungi examined in the present work.

On ordinary broth peptone agar it forms colonies of a deep cinnabar red which, at the outset, adhere strongly to the medium but otherwise differ essentially from the colonies formed by Group I fungi. On other media the colonies may assume other tinges; on water agar, for instance, they become early brownish-black.

According as the colonies grow, the surface becomes more and more irregular, and while the colonies of Group I spread along the plate, sloping evenly in all directions, those of Streptothrix Chalceae rise steeply from the medium in »humpy«, walnut-kernel-like, slightly granular, convolutions. The somewhat larger colonies are more pasty of consistence, and, the larger they grow, the softer their consistence.

Macroscopically, aerial mycelium is never seen. In broth, which remains clear, Streptothrix chalceae grows as small, red, firm, isolated, somewhat irregular spheres on the bottom and some way upwards along the glass of the tube, but *it never exhibits surface growth on liquid media.*

Examining microscopically a subculture from a few days' old culture, we shall see that the surface of the medium is covered by minute, oval, extremely highly refractive bodies which, in the course of some few hours, begin to germinate. Otherwise, the formation of the mycelium takes place in the usual manner, as may be seen in Fig. 63, showing, partly some few spores in their initial stage of sprouting, partly a spore with its young mycelium.

The mycelium is thinner than usually seen in Group I fungi.

After a few days' growth on water agar a peculiar sporulation takes place, *without the formation of any aerial mycelium*, initiating centrally in the colonies. *In this fungus the spores are constantly situated singly at the tip of a usually short side-branch*, which frequently is a little thicker than the main mycelial stem. See Fig. 64.

The mycelium never divides by septa formation, nor do septa arise at the base of the spores. The extreme fineness of the threads of this fungus renders it impossible to detect any differentiation on

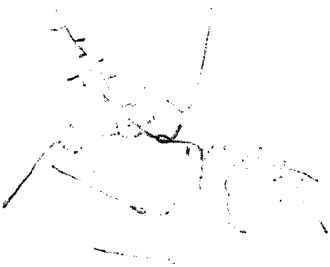


Fig. 63.
Streptothrix Chalceae. 24 hour-culture on water agar, at 37°. Immersion lenses. × 1000.

Fig. 64.
Streptothrix Chalseae. 48-hour culture on water agar, 37°. × 1000

the part of the mycelial protoplasm. That a disintegration does take place, is evident from the fact that, in somewhat older cultures, it is seen how long stretches of the mycelial filaments have become invisible, while the spores lie typically arranged.

The small side-branch, however, on which the spore is situated, will often be seen to grow gradually thinner at the extremity near the spore, so that it is supposedly by a sort of constriction process that the spore is parted from the mycelium. The older the colony grows, the more will the process of spore formation spread towards the periphery. Fig. 65 represents a peripheral branch of a 7 days old water agar colony.

The course of development is the same on agar to which is added glucose and glycerine, just as the microscopic appearance of the fungus when cultivated in broth, is in all details identical to the one on solid medium. Thus, the morphology of the fungus is,

briefly stated, the following; *Formation of a unicellular mycelium which forms distally placed, singly situated spores. No aerial hyphæ. No surface growth in liquid medium.* The fungus absolutely resists desiccation for at least 8 months. Comparison between the power of resistance of the mycelium and the spores, respectively, will no doubt present great difficulty, because it is almost impossible to ensure that the two constituents are actually detached. Otherwise, the mycelium of Streptothrix Chalceae is but slightly

Fig. 66.
Streptothrix Chalceae.

capable of germinating, which may be ascertained by inoculating a water agar plate liberally with a mixture of mycelial threads and spores. While practically all the spores germinate, I have never seen the mycelial threads form new colonies.

I have not seen the above described ray fungus mentioned in the literature, nor have I encountered other fungi with identical morphology; however, the Actinomyces monosporus described by *Schütze*, seems to present a similar mode of forming spores.

In *Lieske's* work some thermophilic fungi are mentioned, which are said to form spores that are situated singly on the outsides of the aerial hyphæ. The fungus in question here cannot however be directly compared with these, as it does not form aerial hyphæ.

Furthermore, *Lieske* mentions (p. 79, Fig. 62) that Actinomyces polychromogenes (Kràl) (which is otherwise described on p. 18 as non-sporogenic) —, one of the fungi subjected to closer examination in a preceding chapter, — may form similar spores when cultivated in glucose broth. It may be that the said fungus can present pictures like that of Fig. 52 in *Lieske's* work, since it is extremely polymorphous, but, that there should be a question of spores, I greatly doubt. At any rate, *Lieske's* fungus bears no resemblance whatever to Streptothrix Chalceae. (This fungus has however also been examined by *Lieske*, but not much commented upon; P. 18 it is descriped as non-sporogenic).

THE MORPHOLOGY OF THE RAY FUNGI IN THE ANIMAL ORGANISM

Before concluding the present work I would consider it appropriate to mention the growth-forms presented by the various ray fungi in the animal organism.

This is of special interest because many of the microbes which are usually reckoned with the true bacteria will, when occurring as parasites, either in natural or artificially produced affections, present forms that are more or less suggestive of the forms usually displayed by the ray fungi; and, the greatest importance has been attached to these relations for the classification of the microbes in question.

The few animal experiments performed by the author have given but sparse information; with our thorough knowledge of the morphology of the cultures in mind, it might however be of interest to review the literature in reference to this matter.

Taking *J. Israel's, Bostroem's* and *Wolff & Israel's* works as starting points, we shall see that all these authors describe their fungi found in the diseased tissues as radiating into all directions from a centre. The fungi consist of a fine network of branching filaments, which are Gram-positive. Surrounding the peripheral ends of the threads, a peculiar refractive substance is frequently found, enveloping the threads like a coat, their mass gradually diminishing from the periphery towards the centre. These threads thereby assume the form of bulbs of clubs. These are commonly termed »Actinomycosis clubs«. In contradistinction from the mycelium, these sheaths are Gram-negative and partly acid-fast.

These ray fungi thus consist of a central network of radially ar-

ranged filaments, at the tips of which the said clubs are frequently found.

Originally it was thought that these forms were absolutely specific for the ray fungi which produce actinomycosis in cattle and man. *Vincent's* Madura fungus, too, exhibits similar clubs, if however not so big.

The above conception has however in the course of time undergone an essential revision.

Already the above-named authors, and later, *Harbitz & Gröndahl, Wright,* and others, showed that it is far from all the actinomycotic nodules that contain clubs, and that the individual cases of disease may vary considerably on this point; *Loele,* for instance, showed that the clubs were of relatively rare occurrence; and, finally, it has proved that actinomycocis-resembling cases produced by ray fungi, occasionally show no clubs at all, while, on the other hand, true ray fungi which are not the causative agents of clinical actinomycosis, may experimentally produce nodules in which the mycelium and the club forms cannot be distinguished from those we encounter in »genuine« actinomycosis. This has for instance been demonstrated by *Nakayama* by animal experiments with Actinomyces Eppinger.

Still more strange does it seem, however, that various authors have encountered cases of clinical actinomycosis which proved to be produced by fungi that had apparently nothing whatever in common with the ray fungi. Thus, as the etiological agent of a nodulous affection in cattle in South America, *Lignières & Spitz* found a small Gram-negative bacillus, which did not display any typical arrangement in the nodules, but was nevertheless surrounded by a fine regular corona of clubs, which in every respect reacted like actinomycosis clubs.

Similar microbes have been found several times, for instance by *Pinoy,* and, in more than half of the cases of a very large series of examinations *Griffith* found Gram-negative bacteria very similar to the *Lignières-Spitz* microbe, so that *Pinoy* seems perfectly right in holding that *Wright* draws too wide conclusions from his investigations of cases of actinomycosis in which fungi of the *Wolff-Israel* type were found exclusevely as etiological agent, inasmuch as he concluded that actinomycocis can be produced by this one fungus only.

Klinger too, in several cases found Gram-negative bacteria of the *Lignières-Spitz* type.

Margrou found that the Staphylococcus which produces botryomycosis, but which is otherwise extremely divergent from the ray fungi, was able to form a »crown of clubs« just like the ray fungi. As for the histological picture, he writes: »L'examen histologique montre l'existence de nombreux grains botryomycotiques, constitués, de même que dans la botryomycose spontanée, par des amas de microcoques entourés d'une coque réfringente; mais ici, la coque périphérique, au lieu d'être homogène comme chez le cheval, est formée d'une palissade de massues identiques par leur forme, leur disposition et leur réactions tinctoriales, aux massues des grains d'actinomycose«.

From this finding *Margrou* draws some — in my opinion rather airy — inferences, which I cannot forbear to quote, because they are to a certain degree typical of the analogisms in which classifiers of bacteria seem inclined to revel:

»La propriété que possèdent les Cohnistreptothrix de fragmenter leur mycelium en éléments isodiamétriques, montre de même que la coupure entre les microbes filamenteux et spheriques n'est pas aussi irreductible qu'on pourrait *a priori* le croire«.

One should not thus draw conclusions from one isolated, apparently common, morphological feature as to the presence of other common characters of which nothing definite at all can be known. In the same work of *Margrou's* there are several other analogical inferences which are still more hazardous than above named.

As already referred to, a number of microbes, the cultures of which we have seen to display certain points of similarity with the Actinomyces, are able to produce forms within the animal organism that are strongly suggestive of those produced by the ray fungi.

Klein showed early that certain strains of diphtheria bacilli, when inoculated subcutaneously in calves, grew into long branching threads in the subcutaneous tissues; and later, *Feistmantel* showed that Actinomyces farcinicus formed similar long branching threads, when the fungus was inoculated into guinea-pigs. True actinomycotic clubs have not as yet been demonstrated in these fungi, whereas *Friederich* and *Babes & Levaditi* have demonstrated that Bacillus tuberculosis is able to produce such when inoculated into rabbits.

For his experiments *Friederich* employed intraarterial injection

of a bacterial emulsion into left ventricle, introducing a long cannula into left arteria carotis communis. By this method he obtained infection of all the organs, and in the nodules arising in the parenchymatous organs, he showed that Bacillus tuberculosis often assumed the forms of a branching mycelium, which was outwardly furnished with clubs that could not be distinguished from actinomycotic clubs.

Unfortunately, the treatise accessible to me has no illustrations, so that it is difficult to form a clear idea of the appearance of the microbes.

In *Babes & Levaditi's* work, there are however very beautiful illustrations from which appears that there is a true mycelial arrangement of the threads, which end in clubs just as in the ray fungi. These authors used to inoculate the tubercle bacilly directly subdurally.

These fine experiments have been repeated from various sides, more thoroughly by *Lubarsch & Schulze*, who undertook to investigate the morphology in the animal organism displayed, partly by the tubercle bacillus, partly by various acid-fast rods, and some few reliably identified ray fungi. The technique applied was the same as that of preceding authors, and the results arrived at also, partly, the same.

When we use the term »partly«, it is due to the fact that the pictures found by *Lubarsch & Schulze* show some divergency from those of *Babes & Levaditi*.

For, on looking closer at the beautiful and distinct pictures which accompany *L. & S.'s* work, we shall find, true enough, a more or less pronounced corona of clubs surrounding a central area of microbes, *but these clubs are not situated in any mycelium with radially arranged threads.* Whereas in the nodules produced by Eppinger's ray fungus, we find this arrangement of mycelial filaments around a centre.

Another objection to be made to these pictures of Actinomyces Eppinger, is that the clubs and mycelial threads show equal susceptibility to staining, which is not true of the mycelium and clubs such as we find them, for instance, in *Wolff-Israel's fungus.*

There is however the possibility that the former clubs are not at all analogous with the latter, but that they are in reality identical with those we know from the cultures on artificial media, and which presumably have nothing to do with »actinomycosis clubs«.

For it is a fact that certain of the ray fungi may form both kinds of clubs when inoculated in animals, *partly clubs which represent swollen areas of the very mycelial filaments, such as we know well from the cultures, and partly clubs that are located outside the threads, such as are found within the animal organism only (Bostroem's and Wright's* statements as to clubs of the latter kind obtained by cultivation of ray fungi on serum-containing media, are no doubt due to a confusion of these two kinds of clubs).

In his interesting and elaborate experiments with Actinomyces Eppinger, *Nakayama* showed, for instance, that the mycelial filaments of this fungus often terminates in large regular clubs which, in respect of staining susceptibility, are identical with the other parts of the mycelium, (See Fig. I, Table VII), while on the other hand, the same fungus may present club formations identical with true »actinomycosis clubs«.

His hypothesis that there should be a connection between these two forms of clubs, the former passing into the latter, does not seem tenable.

This confusion of what might be termed »culture clubs« and »lesion clubs« we encounter in the literature in many authors, the fact that these two kinds of clubs have not been sharply distinguished between having no doubt contributed to make the entire question more intricate.

We have now seen that highly differing microorganisms are able to produce a »corona of clubs« in the animal organism, *so that the isolated demonstration of such is not a sufficient basis for making the diagnosis Actinomycosis, if this be taken to mean an affection produced by ray fungi.*

Furthermore, *Lubarsch* undertook an experiment with inoculation of killed tubercle bacilli in order to show that the club formation is not a purely passive process in which the living bacilli do not participate, finding *in one single case that clubs were actually fomed also by dead tubercle bacilli, if however very small clubs. Lubarsch* does not feel inclined to attach any great importance to this finding. In my opinion, it is however extremely interesting, because we know from several works that such clubs, quite similar to actinomycosis clubs, may arise »passively« in the animal organism. *Coppen Jones* was the first to find such in tuberculous caverns, showing that they formed around elastic threads, and, in a fine work by *Vilh. Jensen*, we find an elaborate description of the

same forms, which are of common occurrence in tuberculous caverns. *Vilh. Jensen* is no doubt right in conceiving them as being produced by deposit of fat stuffs originating from the partly dissolved tubercle bacilli.

The fact that we may thus have »club formations« without live bacteria being necessary for their occurrence, and also, that apparently widely differing fungi are able to produce such, should warn us to be extremely wary in our estimation of these clubs as being of differential value for classification of the fungi in question. It is of far greater interest, with a view to relationship, to demonstrate whether the fungi are able to grow as long branching filaments, or to form a true asteriated mycelium.

Abbot & Gildersleeve performed a similar investigation as *Lubarsch and Schulze*, finding that *Moeller's* grass bacillus, and Bacillus of Thimothy, were able to grow out into a true radiating mycelium in the animal organism. This mycelium frequently terminated in slightly swollen areas, which in regard to staning susceptibility behaved like the mycelial filaments, being presumably identical to the clubs we know from cultures. In numerous colonies the fungi grew as irregular threads and rods, without these being radially arranged. Thus we have here growth in colonies, which are partly build up by a branching mycelium, partly by elements having no such arrangement, just as found in cultures.

Mayer & Minder found in animal experiments that various acid-fast rods and Bac. tub. gallinarum, respectively, grew into asteriated mycelium.

My *own animal experiments* are but few in number and have given no new information of any kind.

I shall just briefly mention that I undertook some experiments with *Streptothrix marked 1/20*, because I thought it possible that the peculiar formations displayed by this fungus on water agar might somehow or other be associated with the club formation in the animal organism.

3 rabbits were inoculated, respectively subcutaneously and intraperitoneally, and, intravenously and intracardially; by the latter mode of injection a general distribution all through the organism is attained far more readily than by the technique applied by *Friederich*.

All the experiments showed negative results, as the injected spores did not produce any lesion whatever. Neither to guinea pigs or white mice, did this fungus prove pathogenic.

A series of experiments were also made with *Actinomyces Sabrazès Rivières.*

One rabbit was inoculated, partly directly into hepar, partly intraperitoneally. After 12 days it was killed without having displayed any disease symptoms worth mentioning. In hepar were found some few firm whitish nodules, in which the fungus was found situated as long granulated threads without any typical arrangement and without clubs. Cultivation from the nodules gave growth of Act. Sabr. Riv.

One rabbit was inoculated intracardially. It showed no distinct symptoms of disease. Killed at the end of 3 weeks. The cannula must supposedly have just missed cor, since the entire pericardium was transformed into an aggregation of larger and smaller nodules, and also left pleural cavity was overgrown by such. Inside the organs there were however no lesions. In the nodules the fungus was found as long granulated threads without typical arrangement; apparently no true growth had taken place. (Such nodules, produced without growth of the infected microbes, is a feature well known from otherwhere. For instance, by injecting killed tubercle bacilli into a guinea pig we may produce a pathologico-anatomical picture that is undistingushable from the one produced by live bacilli). ..

Clubs were not found. Cultivation from the nodules gave growth of the fungus, and of this microbe alone.

Experiments with some few more rabbits likewise produced nodules, if however more sparsely. Otherwise the pictures were identical.

(One might perhaps here have tried to make the fungus more virulent by repeated passages from rabbit to rabbit. In this way *Kolle & Schlossberger & Pfannenstiel* have recently obtained fine results with various acid-fast rods, which were slightly virulent at the outset, but gradually increased in virulence by the passages, to become finally highly virulent, like Bac. tuberculosis).

Inoculations into rabbits, guinea pigs, and white mice, were also performed with *Actinomyces rubra.*

Some few of the mice died after the lapse of a few days. In peritoneum, into which injection was made, some serous exudate and some fibrin coatings on the intestines were found. In these coatings the fungus was situated as minute, almost coccoid, elements. Apparently no growth had taken place.

Cultures were easily obtained from peritoneum. Rabbits and guinea pigs showed no pathological alterations anywhere.

Similar results were attained with *Actinomyces polychromogenes*. Rabbits and guinea-pigs showed no alterations and were apparently totally unacted upon by the microbes. Here, as in the case of Act. rubra, some exudate was found in peritoneum. The fungus seemed not to have grown here; microscopically it proved to consist of short segments. Cultivation experiments gave profuse growth of Act. polychromogenes.

From the literature it is evident that the demonstration of clubs in the animal organism is insufficient basis for making the diagnosis ray fungus affection, and, on the other hand, that the absence of clubs does not preclude the presence of true ray fungi. *Of greater significance for determining the nature of the disease is the demonstration of a branching asteriated mycelium*, the demonstration of such being also of greater importance as regards the question of the systematic position of the fungi.

Furthermore, it is evident that the question concerning the different forms of actinomycosis have not as yet been sufficiently elucidated, so that it is still of the greatest interest that the cases coming under hand be subjected to as thorough pathologico-anatomical investigation as possible; nor should a thorough bacteriological investigation be omitted.

It might very well be conceivable that in several of the cases in which the authors believe to have failed in their attempts at obtaining growth of the pathogenic microbe isolated from actinomycotic affections, the reason has been that the microbe appeared as a ray fungus in the animal organism, while in the cultures it showed bacillary shape, at any rate when examined by the current methods.

SYSTEMATIC POSITION AND NOMENCLATURE OF THE RAY FUNGI

In the present work a classification has been undertaken on the basis of constant morphological characters of the fungi which go under the generic name of Ray Fungi.

As has been seen, this genus included microbes that morphologically were highly different, so different indeed, that it will be difficult to set up definite characteristics as criteria common for the whole group.

On the one hand, we find fungi which present no points of resemblance whatever to the microbes that are usually considered as bacteria, and on the other hand, we have fungi which are now usually classified with the bacteria in the botanical system.

As for the whole group we may say that, systematically, it forms a connecting link between the Hyphomycetes and the Bacteria.

I find it futile to begin speculating upon which of these groups it is most closely allied to.

We divided the fungi into three groups, and found that the Corynebacteria and Mycobacteria investigated in *the present work* must be included in Group II b.

Now, as for setting up a reasonable botanical nomenclature for these groups, this is an extremely difficult task.

As we have already several times had occasion to mention in the preceding sections, the greatest confusion has prevailed just on this point from the very outset. This is no doubt partly due to the fact that, already before success had followed the attempts at cultivating the fungi, these had been denoted by two different designations — those two which up to the present day are most commonly applied, namely: Actinomyces and Streptothrix.

As for both these names obtained that they had already long ago been applied to fungi belonging to utterly differing groups. This made many bacteriologists decide for the rejection of these designations and try to clear the matter by creating new names. Unfortunately, it proved several times that the. new names were beset with similar drawbacks, inasmuch as they had likewise previously been applied to other groups of fungi. Other investigators held to the old terms, taking for granted that confusion could not occur (ordinary conservatism has, no doubt, also played in here).

Another thing too, which has no doubt contributed to make a mess of this question, is that several mycologists, who have undertaken to give names to ray fungi, have not been sufficiently familiar with their morphology. This is true for instance of *Trevisan,* when he proposes the name of Nocardia as a common designation for the ray fungi, on the basis of Nocardia farcinica found by Nocard.

As has been shown, Nocardia farcinica cannot be used as prototype for the whole group. (We saw the difference between Nocardia dassonvillei (Group I), Nocardia Eppinger (Group II a) and Nocardia farcinica (Group II b).

Sauvageau & Radais commit another error by correlating the ray fungi (of Group I) discovered by them, with a previously known group of ray fungus, Oospora, with which they have nothing in common.

There might be stated a number og similar examples from the literature.

In the course of times numerous attempts have thus been made at clearing the question of nomenclature concerning the Ray Fungi, with the result that the problem becomes more and more intricate as time goes, and, taking a quite recent work by *Breed & Cohn,* who have staked a great amount of work on attempting to unravel the entanglements of the Gordian knot, — which the nomenclature question must be said to represent, — the difficulty of the task becomes conspicious. For, even though one be rather familiar with the different names which these authors handle most admirably, it may happen that one becomes a little giddy under the study of their work. Their disentanglement of the question is almost suggestive of a dramatical family law-suit, in which the authors, with the skill of barristers, try to do equal justice to everyone! Nor can the authors be blamed that so poor a positive result ensues from their endeavours. — I shall absolutely forego an attempt at penetrating

deeper to the bottom of the question than has been done by *Breed & Cohn*. I feel convinced, on the whole, that it is impossible to come to any satisfactory settlement of this problem by the roads previously tracked.

The only possible solution is, in my opinion, to cut the knot, and give the different groups names, which, partly cannot be confused with others, partly should be practical in the sense that there is hope of having them generally adopted.

The classification below should of course be conceived as a proposal only, which, it is however my hope, may prove applicable.

The fungi of Group I, I would propose to call Cohnistreptothrix (Streptothrix alone has occasioned confusion with Streptothrix Corda. Cohnistreptothrix, however, cannot involve any misunderstanding).

Group II fungi, I would call Actinomyces (This designation has never caused misunderstandings and the term has presumably become so inveterate that it cannot easily be eradicated.

For Group III fungi, I would propose the name Micromonospora. As has been said, these names, in the sense here given, should only be taken as a proposal from my side.

Of far greater importance than the nomenclature, is that, in future works, we may get as thorough morphological descriptions as possible, so that it may become feasible to determine to which group the fungi described, belong. (Most probably it will prove necessary to set up several new groups).

It is my hope that the present work may contribute to render future morphological investigations easier and more reliable.

RÉSUMÉ

I.

I. In the Introductory Remarks an account is given of how the limits that had been drawn at an early date of the bacteriological era for classification of bacteria on a morphological basis, were rather narrow. A number of works appeared, dealing with divergent bacterial forms which it proved difficult to fit into the once adopted narrow system.

These divergent forms were, and are as yet, most commonly interpreted as being due to unfavourable environmental conditions, and they are usually designated »involution forms«.

The author had earlier been engaged in the study of such divergent bacterial forms and had thereby derived the impression that the term »involution forms« is inadequate, because the various divergent elements found are no doubt of a very different nature.

The so-called »branching bacteria« assume a special position within these divergent forms. It was early found that various bacterial species were able occasionally form genuine side-branches, the nature of which was soon made the subject of lively discussion. Most authors reckoned these branching forms as involution forms, while others conceived them as forms incident to the normal development of the microarganism in question, indicating that its systematic position is not among the true bacteria but among the higher differentiated fungi.

If this were the case, a loss of certain morphological features must have taken place during the course of life of these various microorganisms (primarily, a loss of side-branches).

My interest for these bacterial ramifications was aroused just

through the demonstration of such a transformation of a richly branching microbe which, after the growth of a few generations on artificial medium, almost totally lost its side branches, so that, morphologically, it became strongly suggestive of the so-called corynebacteria.

As I succeeded in devising a method of technique by which it became possible, step by step, to trace the growth of bacteria on a solid transparent medium, I subjected the mentioned microbe to renewed investigation, comparing it with some other ray fungi which were at my disposal at that time.

This investigation soon proved that it was possible to gain a more profound knowledge of the morphology of the ray fungi by this method than by those usually employed for the study of bacteria, and, as a few orientating investigations showed that constant differences of morphology existed between the various ray fungi, differences which partly had not been demonstrated before, partly had been wrongly interpreted, *I took up the task, partly of attempting to undertake a systematic classification of the ray fungi on the basis of constant morphological characters, partly of undertaking a comparison between these ray fungi and certain of the corynebacteria and mycobacteria which, by many bacteriologists, are reckoned as closely allied to the ray fungi.*

II. *The method referred to, for direct tracing of bacterial growth on the surface of a solid transparent medium, is described.*

The basis of this method is that it is possible distinctly to discern even quite minute microbes on the surface of a transparent medium with high-power dry lenses as well as with immersion lenses. The advantage of which being that it is feasible to follow the development of the colonies from the very outset of their formation, which enables the observer to get a much better survey, partly of the morphology of the individual elements, partly of their arrangement in the colonies, than that afforded by the current methods for the study of bacteria.

III. A short historical introduction is given, with reference to the more important works extant, and an account of some of the causes of the confusion prevailing in regard to morphology and classification of the ray fungi. A more thorough valuation of previous works is postponed to a later section where comparison can be made between the author's own and previous experiences.

IV. The notion, relationship, is made the subject of some discussion (See the text).

V. *A grouping of a series of different ray fungi is undertaken on the basis of constant morphological characters,* partly fungi that have previously been isolated in pure culture by other bacteriologists (some of which have been described in the literature), partly fungi found by the author.

. The fungi examined are divided into 3 groups.

Group I

The fungi of this group form a unicellular mycelium on the surface of solid media. (The mycelium which is situated on and in the medium, is termed the *substratum mycelium,* in distinction from *the aerial mycelium* which is protruding upwards into the air). *This substratum mycelium remains undivided during development.* The only interruption of the continuity of the threads that occurs, is an occasional local distintegration of the mycelial threads. The mycelial area lying peripherally to such a breach may however continue to grow independently.

When the development within a given area of the medium is about to cease, aerial hyphæ arise, emerging from the filaments of the substratum mycelium. The aerial mycelium often displays an annular arrangement in the cultures, its presence being moreover revealed by a powderish appearance and usually white colour.

The point of incidence for the aerial mycelium is demonstrated to be primarily dependent on the nutritive content of the medium, as it occurs earlier on media that are poor in nutritive substances — all other conditions being equal. *Likewise, aerial mycelia are of most constant occurrence on poor nutritive media,* since they may sometimes be totally absent on richer media. To this feature, which is doubtless not sufficiently widely known, we may no doubt ascribe some of the divergencies found in the descriptions of ray fungi belonging to this group.

The threads of the aerial mycelium are always thicker than those of the substratum mycelium, and are frequently distinguished by forming a richly branching network of strongly twisted filaments. The cultures very commonly present a number of highly refractive droplets of fluid, exuded by the aerial hyphæ.

After the lapse of some time, the protoplasm of the aerial hyphæ divides into almost equally sized regular parts, (without any pri-

mary occurrence of septa between the individual elements), *which are afterwards separated from each other by a constriction process on the part of the thread membranes.*

These spherical, oval, or cylindrical small bodies are strongly and homogenously refractive. They show no power of resistance against external influences as compared with bacterial endospores, but, compared with the threads of the substratum mycelium, which are also capable of independent growth, they show considerable resistance to various external influences, so that *they must be conceived as a kind of spores in the following sense: as being bodies of uniform shape with a special mode of formation, and being distinguished by a greater power of resistance than the mycelial threads.*

Larger and smaller granules are often formed in the threads of the substratum mycelium and also in certain of the aerial hyphæ; these granules have however nothing to do with the spores, which is manifested, among other things, by the fact that those threads in which these granules abound prove to have lost their power of germination when transplanted on fresh medium.

In liquid media these fungi exhibit a flaky bottom growth. After long time's expiration the flakes may fill out the whole area of the culture fluid, reaching the surface in this way. A profuse aerial mycelium is now frequently formed, quite identical to that formed on solid media. The mycelia of these flakes remain undivided like the substratum mycelium.

While the morphology corresponds in regard to above-named features, the appearance of the cultures is highly varying according to whether aerial mycelium is formed or not. Moreover, the colour of the aerial mycelia may vary considerably even in the same strains, and therefore cannot be used as distinguishing feature between the different fungi.

The substratum mycelium is firm as cartilage and cannot be removed from the medium without lesion to same.

Group I thus consists of sporogenic fungi, the spores growing into a unicellular mycelium that shows no spontaneous division. Out of this substratum mycelium arises an aerial mycelium which divides by a breaking up of the protoplasm into regularly sized parts, which are separated from on another by constriction on the part of the thread membranes in between the individual elements.

In liquid media these fungi show primarily a flaky bottom growth. Not until the flakes fill up the entire area of the culture fluid and reach the surface, do aerial hyphæ appear, which divide into spores.

b. A comparison is undertaken between own and earlier experiences, showing that some authors have given an altogether good description of various fungi belonging to this group, while the great majority of investigators are not clear upon the true nature of these fungi.

c. Actinomyces Affanassiev has been included in this group, because in the cultures it is quite similar to the other fungi of the group. It is however distinct from these by failing to form aerial hyphæ, whereas it forms branches on the media which divide quite in the same manner as do the aerial hyphæ in the other fungi. In liquid media it grows just as has been described above, without surface growth however. In these cultures the mycelium remains undivided.

Group II.

VI. This group comprises fungi which culturally differ more from each other than do the members of the former group. Morphologically, however, they are altogether identical with one another.

This group is again divided into the subgroups *a.* and *b.* according as the fungi form aerial mycelium or not.

a. The fungi of Group II a. initially form an undivided mycelium, which is distinct from the mycelium of Group I fungi by being usually much more irregular of form: polymorphous. *The first aerial hyphæ have their incidence at a very early stage on all media,* usually while the substratum mycelium is as yet so small as to be invisible to the naked eye. In contradistinction from what was the case in Group I, the aerial hyphæ are undistinguishable from the filaments of the substratum mycelium when examined under immersion lenses, that is to say they do not differ essentially either in shape or diameter. For most of the fungi of this group obtains that the aerial mycelium does not grow to any considerable size so as frequently to be detectable only by microscopic investigation. *After the lapse of some time, both the substratum and aerial mycelia divide into segments by means of septa which, emerging from the thread membranes, arise in the threads. The*

segments formed in this way are extremely varying in form and size, and do not, as the spores of Group I fungi, show any augmented resistance as compared with the mycelial threads. These elements are in the present work termed *final cells*, because they are formed most abundantly at the time when development is about to cease.

In liquid media the bottom growth consists of quite small grains, exclusively, while there is *an early surface growth of scales or pellicles.*

The appearance of the cultures may vary considerably. Some fungi form colonies that adhere strongly to the medium, while others can be easily detached.

b. Group II a thus consists of fungi which form an initially undivided mycelium with an early incidence of aerial hyphæ. Both substratum and aerial mycelia divide spontaneously into segments. In liquid media there is early surface growth. The fungi of this group are, besides, distinguished by a pronounced *polymorphism.*

c. This polymorphism is subjected to a closer investigation which shows that the »atypical« forms, which now usually go under the designation involution forms, occur at all stages in the cultures, participating lively in the development, so that there is no reason whatever to interpret them as being due to deleterious external influences. They must be conceived as being equally normal as the regular thread- and rod-shaped elements we find in the cultures.

d. Group II b. The fungi of this group are distinct from the preceding group by the absence of aerial mycelium. *Like these they form an initially undivided mycelium which later divides into segments.*

Certain of these fungi, however, show a tendency to pass into a different mode of growth, which in the present work has been termed the »angular growth«. This mode of reproduction is identical with the one previously described by *Nocard* for Nocardia farcinica and by *Kruse, Hill,* and others, for Bacillus diphtheriæ.

In regard to a number of the fungi examined, which occasionally form true mycelia, it proved that they might pass into the angular mode of growth to such a degree that this mode of growth was occasionally found to be the predominating feature in the cultures.

This angular growth proved to be a constant character in the

fungi concerned, while in the microbes now usually reckoned as bacteria it is found exclusively in the so-called coryne- and mycobacteria.

In liquid media, the fungi of Group II b form early surface growth.

The way in which the colonies adhere to the solid media, is very varying. Some colonies can be quite easily detached from the medium, which especially is true of the strains that have passed into the angular mode of growth.

Group II b thus consists of fungi which, partly may grow into true mycelia, later dividing into segments, partly may display angular growth. In liquid media there is early surface growth.

e. Comparison with earlier works shows that earlier investigators, too, have in some few cases found a transition from richly branching fungi to microbes of rod-shape, of which they frequently remark that they are arranged like diphtheria bacilli in the preparations. There can be no doubt that, in these cases, there has been a question of transformations analogous to those more elaborately described in the present work.

VII. Furthermore, a comparison is undertaken with a number of corynebacteria and mycobacteria, in order to find out whether correspondence in morphology can be demonstrated between these microbes and certain ray fungi.

These investigations proved that the correspondence in morphology is so great that it is not possible to set up any definite boundary between the bacteria examined and those ray fungi which exhibited the angular arrangement of the elements; among other things, the angular mode of growth proved to be as constant a phenomenon among these bacteria as among the ray fungi.

VIII. Among the fungi studied, *Streptothrix chalceae* forms an independent group, Group III.

This fungus forms a unicellular delicate branching mycelium, at the extreme tips of which singly situated, oval spores are formed.

In liquid media it grows as small firm grains at the bottom and upwards along the glass of the tube *without forming any surface growth.*

IX. *a.* Some few inoculation experiments have been performed, without yielding however any new results.

b. As for the review of the literature dealing with the growth-

forms of ray fungi in the animal organism, I shall refer to the text.

X. Finally the questions of nomenclature and systematic position of the fungi are touched upon. The terms: *Cohnistreptothrix* for Group I fungi, *Actinomyces* for Group II fungi, and *Micromonospora* for Streptothrix chalcease and similar fungi, are proposed.

RÉSUMÉ

I. I de indledende Bemærkninger gøres der Rede for, hvorledes der tidligt blev opstillet snævre Grænser i den morphologiske Bakteriesystematik. Imidlertid fremkom der en Mængde Arbejder, som omhandlede afvigende Bakterieformer, som daarligt lod sig passe ind i det een Gang opstillede snævre System.

Disse afvigende Former opfattedes og opfattes i Almindelighed som fremkaldte af ydre ugunstige Livsvilkaar og betegnes sædvanligvis som Involutionsformer. Forfatteren har tidligere beskæftiget sig en Del med saadanne afvigende Bakterieformer og derigennem faaet det Indtryk, at Betegnelsen Involutionsformer er uheldig, idet der sikkert er stor Forskel paa forskellige afvigende Formelementers Natur.

En særlig Stilling indenfor disse afvigende Former indtager de saakaldte »forgrenede Bakterier«. Det viste sig tidligt, at forskellige Bakterier var i Stand til nu og da at danne ægte Sidegrene, og disses Natur blev snart Genstand for livlig Discussion. De fleste regnede dem for Involutionsformer, medens andre opfattede dem som normale Udviklingsformer, der fortæller os, at de paagældende Mikrober egentlig ikke hører hjemme blandt Bakterierne i systematisk Henseende, men mellem i morphologisk Henseende højere differentierede Svampe.

For at dette skulde være Tilfældet, maatte der under disse forskellige Microorganismers Livsløb have fundet et Tab af visse morphologiske Træk Sted. (Først og fremmest et Tab af Sidegrenene).

Min Interesse for disse Bakterieforgreninger blev vakt netop gennem Paavisningen af en saadan Omdannelse af en rigt forgrenet Mikrob, som efter faa Generationer paa kunstigt Substrat mistede Sidegrenene næsten fuldstændigt og i morphologisk Henseende kom til at minde stærkt om de saakaldte coryneforme Bakterier.

Da det nu lykkedes mig at udfinde en Metode, ved Hjælp af hvilken det var muligt at følge Væksten af Bakterier paa fast, gennemsigtigt Substrat Trin for Trin, tog jeg den omtalte Svamp op til fornyet Undersøgelse og sammenlignede den med nogle andre Straalesvampe, som paa det Tidspunkt stod til min Disposition.

Ved denne Undersøgelse viste det sig hurtigt, at det var muligt at trænge dybere til Bunds i Straalesvampenes Morphologi end ved de sædvanlige Bakterieundersøgelser, og da der efter nogle faa orienterende Undersøgelser viste sig at bestaa konstante morphologiske Forskelle mellem forskellige Straalesvampe, som dels ikke var konstaterede tidligere, dels tydede urigtig, *tog jeg den Opgave op dels at gøre et Forsøg paa at foretage en systematisk Inddeling af Straalesvampene efter konstante morphologiske Egenskaber, dels at foretage en Sammenligning mellem disse Straalesvampe og visse af de coryneforme Bakterier og Mycobakterierne, som af mange Bakteriologer regnes for nøje beslægtede med Straalesvampene.*

II. *Den omtalte Metode til direkte Forfølgelse af Bakterievæksten paa Overfladen af fast gennemsigtigt Substrat beskrives.*

Grundlaget for denne Metode er dette, at det er muligt at se selv smaa Mikrober tydeligt paa Overfladen af et gennemsigtigt Substrat saavel med et stærkt Tørlinsesystem som med Immersionssystemet. Man opnaar herved at kunne følge Udviklingen i Kolonierne fra første Begyndelse, og derigennem bliver man i Stand til at faa et langt bedre Overblik dels over de enkelte Elementers Morphologi, dels over disses Lejring i Kolonierne end de sædvanlige Bakterieundersøgelsesmetoder giver.

III. Der gives en kort historisk Indledning, i hvilken de vigtigste Arbejder omtales, og i hvilken der gøres Rede for nogle af Grundene til den Uklarhed, der har hersket i morphologisk og systematisk Henseende. Den nøjere Vurdering af de forskellige tidligere Arbejder opsættes til et Tidspunkt, hvor der kan foretages en Sammenligning mellem egne og tidligere Erfaringer.

IV. Begrebet Slægtskab gøres til Genstand for en lidt nøjere Behandling. (Se Teksten).

V. *Paa Basis af konstante morphologiske Egenskaber foretages der dernæst en Gruppeinddeling af en Række forskellige Straalesvampe,* dels saadanne, der er rendyrkede tidligere af andre Bakteriologer (nogle af dem er beskrevne i Literaturen) dels saadanne, jeg selv har fundet.

a. De undersøgte Svampe inddeles i 3 Grupper.

Gruppe I.

Svampene i denne Gruppe danner et eencellet Mycel paa Overfladen af faste Substrater. (Mycelet, som ligger paa og i Substratet kaldes i Modsætning til det op i Luften ragende Luftmycel, *Grundmycelet). Dette Grundmycel forbliver udelt under Udviklingen.* Den eneste Afbrydelse af Traadenes Kontinuitet, der opstaar, fremkommer ved, at Mycelets Traade af og til kan falde hen paa et Stykke. Det perifert for Afbrydelsen liggende Mycelparti kan vokse videre selvstændigt.

Naar Udviklingen paa et givet Parti af Substratet er ved at nærme sig sin Afslutning, opstaar der Luftgrene, som stiger op fra Grundmycelets Traade. Luftmycelet danner ofte Ringe i Kulturerne og røber deres Tilstedeværelse ved sin pudderagtige, oftest hvide Farve.

Det paavises, at Tidspunktet for Luftmycelets Optræden i første Række er afhængigt af Substratets Næringsholdighed, idet det optræder tidligst alle andre Forhold lige paa saadanne Substrater, som er fattige paa Næringsstof. *Luftmycelet dannes ogsaa sikrest paa næringsfattige Substrater,* idet dets Dannelse af og til kan udeblive paa rigere Substrater. I dette Forhold, som sikkert ikke er tilstrækkelig kendt, findes sikkert een af Aarsagerne til de afvigende Beskrivelser af Straalesvampene hørende til denne Gruppe.

Luftmycelets Traade er altid tykkere end Grundmycelets og udmærker sig ofte ved at danne et rigt forgrenet Netværk af stærkt snoede Traade. Meget ofte ser man i Kulturerne en Mængde stærkt lysbrydende Vædskedraaber, som udsvedes af Luftmycelets Traade.

Efter nogen Tids Forløb deler Protoplasmaet i Luftmycelets Traade sig i næsten lige store regelmæssige Stykker (altsaa uden at der primært opstaar nogen Skillevæg mellem de enkelte Elementer), som senere adskilles indbyrdes, ved at Traadenes Sidevægge snører sig ind mellem de enkelte Elementer.

Disse runde, ovale eller cylindriske Smaalegemer er stærkt og ensartet lysbrydende. De viser ingen Modstandsevne overfor ydre Paavirkninger som Bakteriernes Endosporer, men viser i Sammenligning med Grundmycelets Traade, som ogsaa er i Stand til selvstændig Vækst, en betydelig Resistens mod ydre Paavirkning af forskellig Art, saaledes at *de maa opfattes som en Slags Sporer i den følgende Betydning: Indbyrdes i Form overensstem-*

mende Legemer, der dannes paa en særlig Maade og udmærker sig ved en større Modstandsdygtighed end Mycelets Traade.

I Grundmycelets og visse af Luftmycelets Traade dannes der ofte større og mindre Korn, som imidlertid intet har med Sporerne at gøre, hvilket blandt andet viser sig derved, at de Traade, der er rigeligst forsynede med dem, viser sig at have mistet deres Spireevne paa nyt Substrat.

I flydende Substrat vokser disse Svampe som Fnug paa Bunden. Efter lang Tids Forløb kan disse fylde hele Vædsken og naa Overfladen paa denne Maade. Her dannes der saa ofte et rigt Luftmycel af ganske samme Art som paa de faste Substrater. Mycelet i Fnuggene forbliver udelte som i Grundmycelet.

Medens Morphologien er overensstemmende med Hensyn til de ovennævnte Træk, er Udseendet af Kulturerne højst forskelligt væsentligst afhængigt af, om der er dannet Luftmycel eller ej. Farven af Luftmycel kan variere meget selv indenfor de samme Stammer og kan saaledes ikke bruges til Skelnemærke mellem de forskellige Svampe.

Grundmycelet er bruskagtigt haardt og kan ikke fjernes fra Substratet uden samtidig at lædere dette.

Gruppe I bestaar altsaa af Svampe, som danner Sporer, der vokser ud til et eencellet Mycel, som ikke deler sig spontant. Fra dette Grundmycel udspringer der et Luftmycel, som deler sig ved at Protoplasmaet falder hen i regelmæssigt store Stykker, der dernæst adskilles indbyrdes, ved at Traadmembranerne snører sig ind mellem de enkelte Elementer.

I flydende Substrat vokser de i Fnug begyndende paa Bunden. Først naar Fnuggene fylder hele Vædskelaget og naar Overfladen, dannes Lufthyfer, der deler sig i Sporer.

b. Der foretages en Sammenligning mellem egne og tidligere Erfaringer, hvorved det viser sig, at enkelte Forfattere har givet en i det store og hele god Beskrivelse af forskellige Svampe hørende til denne Gruppe, medens det store Flertal af Undersøgere ikke er klare over Svampenes Natur.

c. Til denne Gruppe er desuden regnet en Svamp, Actinomyces Affanassiev, fordi dens Kulturer fuldstændigt ligner de øvrige Svampe i Gruppen. Den adskiller sig imidlertid ved ikke at danne Lufthyfer, hvorimod der paa Substraterne dannes Grene, der deler sig ganske som Luftmycelet hos de øvrige Svampe. I flydende Substrat vokser den ganske som ovenfor beskrevet, uden dog at

danne Overfladevækst. Mycelet forbliver i disse Kulturer udelt.

VI. *Gruppe II.* Denne Gruppe Svampe omfatter Svampe, om i kulturel Henseende er mere forkellige indbyrdes end Svampene i foregaaende Gruppe. I morphologisk Henseende er der derimod i det store og hele Overensstemmelse.

Gruppen underafdeles i Afdeling a og b eftersom Svampene danner Luftmycel eller ikke.

a. *Svampene i Gruppe IIa danner til en Begyndelse et udelt Mycel,* som adskiller sig fra Mycelet i Gruppe I ved oftest at være langt mere uregelmæssigt af Form, polymorpht. *Meget tidligt opstaar de første Lufthyfer paa alle Substrater,* oftest mens Grundmycelet endnu er saa lille, at det ikke kan ses med det blotte Øje. Lufthyferne lader sig i Modsætning til, hvad Tilfældet var i Gruppe I, ikke skelne fra Grundmycelets Traade, naar Undersøgelsen foretages med Immersionssystemet, afviger altsaa ikke synderligt hverken i Form eller Tykkelse. Hos de fleste af Svampene i denne Gruppe naar Luftmycelet kun ringe Størrelse og opdages ofte først ved den mikroskopiske Undersøgelse. *Efter nogen Tids Forløb deler Grundmycel og Luftmycel sig i Segmenter ved at der, begyndende fra Traadmembranerne vokser Skillevægge ind gennem Traadene. De derved opstaaede Segmenter er af meget forskellig Form og Størrelse og viser ikke som Sporerne i Gruppe I foroget Modstandsdygtighed* i Sammmenligning med Mycelets Traade.

I flydende Kulturer dannes paa Bunden af Vædskerne kun meget smaa Korn, hvorimod der *tidligt opstaar Skæl eller Hinder paa Overfladen.*

Kulturernes Udseende kan være meget forskellige. Nogle af Svampene danner Kolonier, der sidder meget fast paa Substraterne, andres Kolonier sidder ganske løst.

b. *Gruppe IIa bestaar altsaa af Svampe, som danner et til en Begyndelse eencellet Grundmycel, som tidligt danner Lufthyfer. Saavel Grundmycel som Luftmycel deler sig spontant i Segmenter. I flydende Substrat opstaar tidligt Overfladevækst.* Svampene i denne Gruppe udmærker sig desuden ved en udtalt *Polymorphi.*

c. Denne Polymorphi bliver gjort til Genstand for en nærmere Undersøgelse, og herved viser det sig, at de »afvigende« Former, som nu sædvanligvis gaar under Betegnelsen Involutionsformer, optræder paa alle Tidspunkter i Kulturerne og tager livlig Del i

Udviklingen, saaledes at der ingensomhelst Grund er til at opfatte dem som opstaaede paa Grund af ydre skadelige Paavirkninger. De maa opfattes som ligesaa normale som de regelmæssige traad- og stavformede Elementer, vi træffer i Kulturerne.

d. *Gruppe II b.*

Svampene i denne Gruppe adskiller sig kun fra foregaaende ved intet Luftmycel at danne. *De danner ligesom disse et til en Begyndelse udelt Mycel, som senere deler sig i Segmenter.*

Visse af disse Svampe er imidlertid tilbøjelige til at slaa over til en anden Vækstmodus, som i dette Arbejde kaldes Vinkelvækst. Denne Maade at formere sig paa er identisk med den tidligere af *Nocard* for Nocardia farcinica af *Kruse, Hill* og andre for Bac. diphtheriae beskrevne Maade at gro paa.

For en hel Række Svampe, som af og til kan danne et ægte Mycel, viste det sig, at de kunde gaa over til Vinkelvæksten, og at denne af og til bliver aldeles dominerende i Kulturerne.

Denne Vinkelvækst viste sig at være ganske konstant hos de paagældende Svampe, medens den hos de Mikrober, som nu regnes til Bakterierne, kun findes hos de saakaldte coryneforme Bakterier og Mycobakterierne.

I flydende Substrater danner Svampene i Gruppe II b tidligt Overfladevækst.

Paa fast Substrat sidder Kolonierne meget forskelligt fast. Nogle er ganske løstsiddende, dette gælder særligt de Stammer, som er slaaet over til Vinkelvæksten.

Gruppe II b bestaar altsaa af Svampe, som dels kan danne et ægte Mycel, der senere deler sig i Segmenter, dels kan vokse med Vinkelvækst. I flydende Substrat dannes tidligt Overfladevækst.

e. Ved Sammenligning med tidligere Arbejder viser det sig, at ogsaa tidligere Undersøgere i enkelte Tilfælde har fundet Omdannelser fra rigt forgrenede Svampe til Mikrober af Stavform, om hvilke de ofte beretter, at de i Præparaterne ligger lejrede som Difteribaciller. Der er ingen Tvivl om, at det ogsaa her har drejet sig om Omdannelser analoge med de i dette Arbejde nøjere beskrevne.

VII. Yderligere er der foretaget en Sammenligning med en Del af de coryneforme Bakterier og Mycobakterier for at undersøge, om der kan paavises Overensstemmelse i Morphologi mellem disse og visse Straalesvampe.

Det viste sig herved, at der er en saa stor Overensstemmelse i Form, at det ikke er muligt i morphologisk Henseende at opstille

nogen Grænse mellem de undersøgte Bakterier og de vinkelvoksende Straalesvampe; blandt andet viste Vinkelvæksten sig at være ligesaa konstant et Fænomen blandt disse Bakterier som hos Straalesvampene.

VIII. En Gruppe for sig, *Gruppe III*, danner mellem de undersøgte Svampe den saakaldte *Streptothrix chalceae*.

Den danner et eencellet fint, forgrenet Mycel, paa hvis yderste Grenspidser, der danner sig enkeltsiddende ovale Sporer. I flydende Substrat vokser den som smaa faste Korn paa Bunden og op langs Glassets Sider, uden at danne Overfladevækst.

IX a. Der er foretaget nogle faa Inoculationseksperimenter, som dog ingen nye Resultater har bragt.

b. Med Hensyn til Litteraturgennemgangen af Arbejderne omhandlende Straalesvampenes Vækstformer i den dyriske Organisme henvises til Teksten.

X. Slutteligt berøres Spørgsmaalet om Svampenes Nomenklatur og systematiske Stilling. Det foreslaas her at anvende Betegnelserne *Cohnistreptothrix* for Svampene i Gruppe I, *Actinomyces* for Svampene i Gruppe II og endelig *Micromonospora* for Streptothrix chalceae og lignende Svampe.

LITERATURE

It is only a minor part of the works extant treating of Ray Fungi that I have included in the list below. It will appear from the work itself on what lines I have made my selection. I have included those papers only which are made the subject of some valuation in the text.

As for some especially early works applies, that they have not been accessible.

It is however my hope that I have succeeded in including most of the works which are of any special interest in this connection.

Investigators who might wish to study the literature on Ray Fungi on a larger scale may be referred to the more comprehensive indexes of literature in the works of *Musgrave & Clegg*, and *Lieske*.

The following abbreviations are used:

C. f. B. = Centralblatt für Bacteriologie und Parasitologie. Original.
Z. f. H. = Zeitschrift für Hygiene.
Annal. Past. = Annales de l'Institut Pasteur.

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